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The Journal of Clinical Investigation

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Contents

The Responses to Painful Stimuli of Patients with Severe Chronic Painful Conditions. MARGARET A. KENNARD 245

Diurnal Variation in the Urinary Excretion of Neutral Lipid-Soluble Reducing Steroids in Congestive Cardiac Failure and Cirrhosis of the Liver with Ascites. RALPH GOLDMAN AND SAMUEL H. BASSETT..... 253

The Effects of Potent Analgesics upon Vestibular Function. LEONARD B. GUTNER, WILBER J. GOULD, AND ROBERT C. BATTERMAN..... 259

The Effect of Theophylline with Ethylenediamine (Aminophylline) on Cerebral Hemodynamics in the Presence of Cardiac Failure with and without Cheyne-Stokes Respiration. JOHN H. MOYER, SAM I. MILLER, ARTHUR B. TASHNEK, AND RUSSEL BOWMAN..... 267

The Effects of 1-Epinephrine and 1-Nor-Epinephrine upon Cerebral Circulation and Metabolism in Man. BENTON D. KING, LOUIS SOKOLOFF, AND RICHARD L. WECHSLER..... 273

The Volume of Distribution of Sodium Thiosulfate as a Measure of the Extracellular Fluid Space. R. H. CARDOZO AND I. S. EDELMAN..... 280

The Electrolyte Content of Skeletal Muscle in Congestive Heart Failure; A Comparison of Results with Inulin and Chloride as Reference Standards for Extracellular Water. REUBEN MOKOTOFF, GEORGE ROSS, AND LOUIS LEITER..... 291

Disturbances of Ionic Equilibrium of Intracellular and Extracellular Electrolytes in Patients with Tuberculous Meningitis. HAROLD E. HARRISON, LAURENCE FINBERG, AND EVELYN FLEISHMAN..... 300

Validity of the Endogenous Creatinine Clearance as a Measure of Glomerular Filtration Rate in the Diseased Human Kidney. BENJAMIN F. MILLER, ALEXANDER LEAF, AUDLEY R. MAMBY, AND ZELMA MILLER.... 309

A Comparison of the Allantoin and Inulin Clearances for the Measurement of Filtration Rate in the Diseased Human Kidney. BENJAMIN F. MILLER, ALEXANDER LEAF, AUDLEY R. MAMBY, AND ZELMA MILLER.... 314

Some Aspects of the Human Pharmacology of Tetraethylthiuramdisulphide (Antabus)-Alcohol Reactions. CHARLES H. HINE, THOMAS N. BURBRIDGE, EDWARD A. MACKLIN, HAMILTON H. ANDERSON, AND ALEXANDER SIMON..... 317

Reversal of Diurnal Variation in Renal Function in Cases of Cirrhosis with Ascites. RICHARD A. JONES, GERALD O. MCDONALD, AND JULES H. LAST..... 326

Studies in Glycine-2-C^{14} Metabolism in Man. II. Tissue Distribution. NATHANIEL I. BERLIN, BERT M. TOLBERT, AND CAROL LOTZ..... 335

The Basal Secretion of Pepsin by the Human Stomach. HENRY D. JANOWITZ AND FRANKLIN HOLLANDER.... 338

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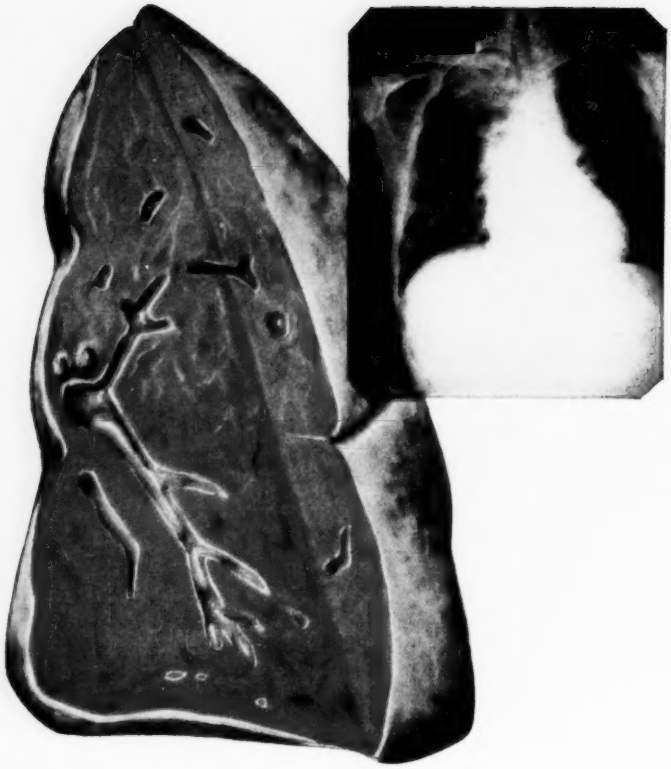

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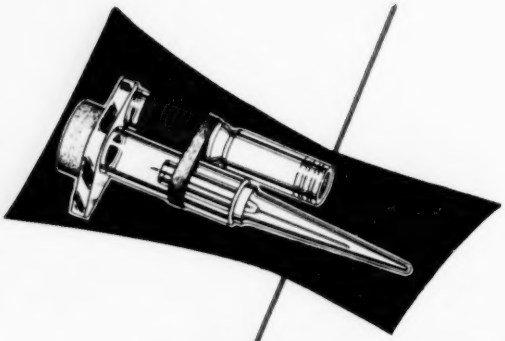

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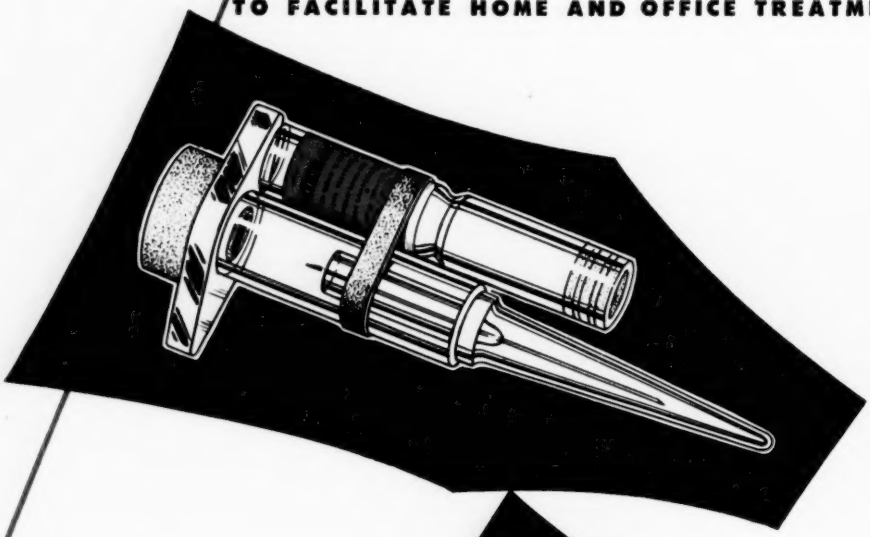

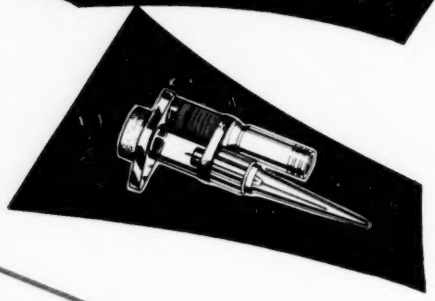

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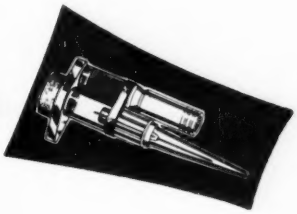

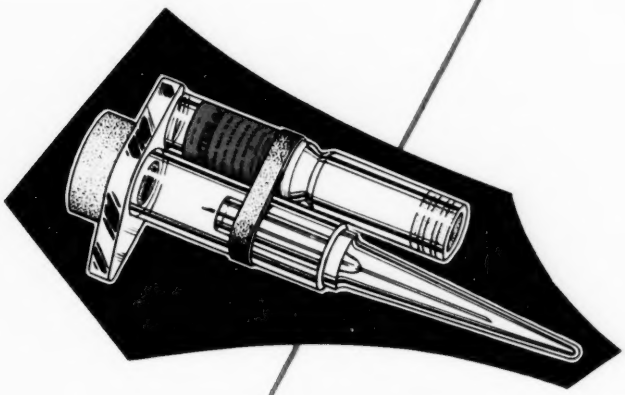

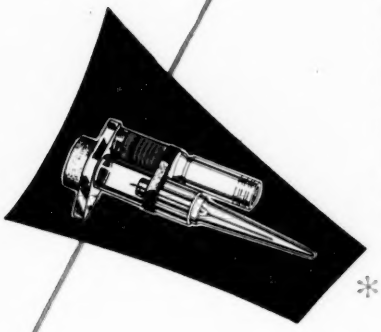

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THE RESPONSES TO PAINFUL STIMULI OF PATIENTS WITH SEVERE CHRONIC PAINFUL CONDITIONS

By MARGARET A. KENNARD [1]

(From the Department of Surgery, University of Oregon Medical School, Portland, Oregon)

(Submitted for publication March 1, 1951; accepted December 10, 1951)

In the study of patients who have severe and prolonged, chronic, painful conditions, it is a clinical fact that some will react more profoundly than others to what are apparently similar conditions. It is also obvious clinically that pain is an extreme fear- and anxiety-provoking stimulus. Furthermore, the diagnosis of "functional pain," or of a "functional element" in pain, is one frequently attached to many of these patients. Because of the confusion generated by these clinical observations it was thought possible that a study of the responses of such patients to painful stimuli might aid in their diagnosis and perhaps add to the knowledge of the physiology of pain.

Such an investigation has been made possible by the recent studies on the physiology of pain which have resulted in a better definition of this very difficult subject. Wolff and his associates have demonstrated that a *pain threshold* can be quantitatively measured (1); that the perception of pain intensities is relatively fixed for a given person under given environmental conditions; and that thresholds for pain perception vary relatively little from person to person (2). They have also described *reaction to pain* which is a more variable factor and is observable as a somatic response on the part of the patient. This *reaction* is related, in their opinion, to bodily and emotional phenomena such as fear, anxiety or apprehension which are widely variable in different people and from time to time in the same person (3).

The present study is an attempt to compare the thresholds and pain reactions of normal subjects with those of patients who have been undergoing severe pain. In this study a third factor has also been examined, namely, the conditioning time to painful stimuli. This part of the project is based on the studies of Welch and Kubis (4) who demonstrated that the conditioning time of neurotic patients with severe anxiety was significantly shorter than that of normal controls.

METHOD

Pain threshold: An electrical stimulus was chosen for the measurement of pain threshold. This was standardized in the Laboratory by tests carried out on staff members prior to the present investigation. A Goodwin Stimulator (model 3) was used which was so set as to deliver a single shock of uniform wave-form (20 sigma falling phase) and of varying voltage intensity. The stimulus strength was always increased in 10-volt units, a gradient which made single unit changes barely perceptible. The stimulus was delivered to the thin-skinned area of the volar surface of the upper left forearm. Contact was made through two small solder bars about 1 cm. long and 3 mm. wide which were fastened parallel and about 3 mm. apart onto a rubber base. The same pair of electrodes was used for all subjects in the present tests. Electrodes were held in place by a rubber strap around the arm such as is used for EKG electrodes. The skin beneath the electrodes had been previously rubbed vigorously with a small amount of electrode jelly.

Galvanic skin response: The reaction to pain was recorded by means of the galvanic skin response (GSR). This reaction is known to result from a sudden change in sweating and to be dependent on arousal or startle in the subject. It was recorded through electrodes attached to the right wrist and palm. One solder electrode, about 1½ cm. in diameter, was pressed against the right palm and a second, about ½ cm. in diameter, against the volar surface of the right wrist. Each electrode was fastened to a rubber base which was round and about 4 cm. in diameter, held against the skin by a strap. Contact was made by rubbing the skin with electrode paste and then covering each electrode with the jelly. If the contact became dry during the procedure, artefacts appeared in the record and additional jelly was inserted between the electrode and the skin. A ground electrode was fastened to the right ear. The GSR was recorded on an eight-channel Grass Electroencephalograph according to the method described by Redlich (5). The machine was run at "slow" speed, *i.e.*, 1½ cm./sec.

Conditioning response: Subjects were conditioned to either painful or auditory stimuli according to the procedure described by Welch and Kubis (4). In order to be sure of comparable findings a duplicate of their film-sequence of nonsense syllables of low association value was obtained from Dr. Welch and his procedure was followed as precisely as possible.

[1] Present address: Department of Neurological Research, University of British Columbia, Vancouver, Canada.

Subjects were asked to read out loud a series of nonsense syllables projected before them, one every six seconds. The unconditioned stimulus was given every other time that a certain syllable (KAX) appeared on the screen. The reaction was recorded as GSR. The painful stimulus used was arbitrarily set at 10 volts more than the threshold just previously determined for the individual. In the group in which sound instead of pain was used for the unconditioned stimulus, the exact procedure of Welch and Kubis was followed. A door-bell placed just behind the subject's chair was rung loudly at the appropriate instant (*i.e.*, every other time that KAX appeared). Conditioning time was recorded as that number of times at which KAX had appeared when the first of three consecutive GSRs appeared at an unreinforced syllable. At least 30 trials were given for each subject.

The subjects: These were 57 normal adult controls and 24 adult patients from the Pain Clinic of the University of Oregon Medical School (Table I). The controls were either: the relatives of patients, volunteers from the second year class of medical students, those from a class of student nurses, or members of the Laboratory staff. The patients all suffered from organic conditions involving severe, prolonged pain of months' or years' duration which was still present. The patients' ages varied from 21 to 65, although only three were over 56, the average age being 42.9. There were 16 females and eight males. Diagnoses were as follows: causalgia-like syndrome, six; low back pain, five; phantom limb pain, four; intra-abdominal pain, three; arthritis, two; chronic cystic pancreatitis, two; and long-standing coronary disease, two. All had been unable to work, and in severe discomfort for months, the primary cause for their disability being pain and not any other symptom coincident to their illness. The existent pain syndrome was present and unabated at the time of the tests, although severe pain was present in only five cases (Nos. 39, 105, 110, 135, 151). Seven had no pain (Nos. 79, 102, 104, 106, 111, 119, 148) and the remainder felt mild or moderate pain during the procedure. All patients were ambulatory and were attending the Clinic at the time of the testing. Six were at least partially dependent on morphine derivatives (Nos. 39, 68, 71, 106, 110, 112). As far as could be ascertained, as previously instructed, none had had any medication for 24 hours preceding the test.

Anxiety factor: An attempt was made to appraise the factor of anxiety in the patients. Every effort was made to make the test situation as little alarming as possible. All subjects were asked if they would be willing to cooperate in a project which was designed to investigate the "reactions of people." It was explained that people differed in this respect and that, although the tests might be no direct help to the present pain of the patient, it might ultimately help in understanding his condition and those of others in like difficulties. Each subject was then assured that the procedure would take only about half an hour; that it would not "hurt" and no needles would be used; that no test given would "bother" the subject; and that our method was to use the amount of sweating to measure various reactions.

Both controls and patients were relaxed and co-operative at the time of the test. One subject (No. 79) expressed the idea after the tests that "waiting for those words" had made him feel as though he might be going to have an attack (coronary). Two (Nos. 39, 50) were slightly resentful at being put through the procedure, although they had agreed previously to co-operate. Without knowledge of the test results a "most anxious" and "least anxious" group were selected by three individuals who knew the patients well (the doctor in the Clinic, the doctor guiding the experimental procedure and the psychologist) and these ratings were later examined in relation to the tests. All but two of the patients—the two with chronic arthritis—had been described earlier as either having "purely functional" pain or at least a "strong functional element" by the doctors who had sent them to the Pain Clinic. There were eight of the 24 who were definitely of the over-reactive type, chronic habitués of all clinics, whose focus of attention in life was undoubtedly upon their somatic ills (Nos. 38, 39, 55, 96, 101, 109, 111, 112). However, all 24 patients had true focal pain of organic origin.

Test procedure

The subject was seated in a comfortable chair facing the projection screen and turned away from the investigator and the recording apparatus. The movie projector was above and behind the chair. The directions given were almost identical for each individual throughout the procedure, the author and one other person being present at each test. Tests were always given in the same sequence.

Test One: The subject was told that this test was "just to try out the apparatus." He was then asked to think of "any number between one and 10 inclusive" and to think about that single number persistently *without replying* to the investigator's queries, the machine being turned on during the procedure. He was asked, "Is it three?," "Is it nine?," etc., etc. Each number was asked twice, the sequence being random but invariable since it was read from a card. The GSR record was then examined. In the event that a marked GSR swing had appeared for a single number the subject was told what his choice had been. This test served several purposes—to be sure that the system was working; to allow calibration of the GSR swing; and to reassure the subject who was usually amused or a little incredulous, according to his sophistication in such matters.

Test Two: Pain threshold. Starting well below threshold, graded stimuli were given from the Goodwin Stimulator. Each stimulus was given twice at about one second's interval and the subject became used to the click of the switch whether a stimulus was felt or not. No GSR recording was made at this time, but the subjective impression of the patient was written on the record. At a given point, as the threshold was increased, the subject felt a touch or tickle ("T" in Table I). He was then told that the stimulus would be gradually increased "like this" and that at a given point he would feel a prick or pain instead of a touch, but that it would never be a pain which

would "make you burst out crying." This threshold for pain ("P" in Table I) was then noted on the record. During this test the stimulus gradient was always increased 10 volts at a time without any variation in the procedure. After the pain threshold had been reached two or three more stimuli were delivered just above, below and at threshold to check its level. The subject was then told that subsequent prick stimuli would never be more intense than those already experienced.

Test Three: For the *conditioning test* the pain stimulator was set at 10 volts above the pain threshold just reported. "I want you to read out loud the syllables which I am going to throw on the screen in front of you. Never mind how you pronounce them. Some are rather hard to pronounce, but just keep on and on reading them while I turn on the machine and make a record." For this procedure the room was partially darkened. All of the patients and about half of the controls were conditioned to a pain stimulus. The remainder of the controls were conditioned using sound instead of pain for the unconditioned stimulus.

Test Four: At the end of the above, the subject was told that there was just one more brief test in which we would recheck the reactions which had been recorded previously, and that he was merely to sit still with eyes closed for a few minutes while we ran over the previous test. The GSR was then recorded during a sequence of stimuli which started at 10 volts and increased 10 at a time. Five stimuli, three seconds apart, were given at each level, until a GSR response was obtained or until the strength of the stimulus was two units beyond that of the previous pain threshold. Since, with no exceptions, a GSR had been obtained in the just-preceding test at a stimulus level only one unit above threshold for pain, it was felt that two units above must be adequate for the present test. GSR threshold was considered to be that strength of stimulus which produced a GSR in three of the five trials, *if* that same change was also produced at the level one unit higher. This last condition was found necessary because, in a few instances, and for no obvious reason, a subject might respond three times to a stimulus at a given level but give no further response at several higher levels. When this happened it was thought that there had been fluctuations in the awareness of the subject resulting in re-enforcement of the applied stimulus.

TABLE I

Threshold levels at which touch (T) and pain (P) were felt and at which galvanic skin responses (GSR) and conditioned reflexes (CR) appeared

Pain was used as the conditioning factor in one group of controls and in all patients. Sound was used in the second control group.

Controls, pain stimuli						Controls, sound stimuli						Patients					
No.	Age	T	P	GSR	CR	No.	Age	T	P	GSR	CR	No.	Age	T	P	GSR	CR
Female						*Female*						*Female*					
40	25	—	50	40	10	53	23	20	50	20	7	35	37	—	40	—	5
45	22	—	70	50	10	54	21	30	50	50	9	38	28	20	60	20	8
46	25	—	40	50	10	56	24	10	20	10	7	39	32	—	80	—	10
47	21	—	80	80	10	58	37	10	30	40	8	55	38	40	90	—	—
59	26	20	70	60	12	67	24	20	70	60	12	57	35	10	40	—	5
63	43	20	80	60	4	76	27	30	60	40	2	90	50	20	50	50	2
77	27	10	80	80	6	125	20	20	50	50	12	96	27	20	50	80	10
81	21	20	90	80	9	126	20	20	50	60	14	102	30	30	60	60	14
98	38	20	70	80	16	127	20	20	60	80	4	104	21	20	80	80	14
103	28	20	60	60	8	128	20	10	40	50	8	109	32	20	60	30	14
107	22	20	60	70	4	129	20	10	60	80	10	110	56	30	70	80	6
108	36	30	80	60	10	130	20	50	80	90	8	111	56	30	70	50	4
134	37	10	60	40	6	133	20	20	60	50	12	112	56	30	80	80	2
137	43	20	60	60	6	138	20	10	50	10	13	119	38	30	70	80	9
141	25	10	40	50	4	139	20	20	60	50	12	121	41	10	40	40	4
Male						142	20	20	70	60	—	151	50	10	30	40	—
42	24	—	80	40	10	143	20	10	60	40	6	*Male*					
43	27	—	90	60	10	144	20	20	70	50	8	68	55	20	60	—	—
48	25	30	80	30	13	145	25	10	50	50	10	71	65	20	90	80	13
60	24	30	80	40	11	146	22	20	70	80	8	79	43	20	50	90	13
62	20	20	80	30	6	147	23	20	80	80	2	101	40	20	80	—	15
69	24	40	70	50	8	*Male*						105	43	50	80	90	15
70	22	30	90	60	8	61	20	40	90	50	4	106	60	20	70	80	2
72	21	10	40	50	—	65	24	30	50	40	18	135	45	40	90	—	6
73	29	40	80	90	14	66	24	30	70	60	26	148	52	10	50	50	16
74	22	30	70	50	12	122	25	20	80	60	6						
75	22	30	60	40	4	123	23	30	80	90	8						
78	24	40	90	80	19	124	26	10	80	70	19						
80	24	10	60	30	10												
132	42	30	80	70	2												
140	26	50	80	50	18												

DATA

In Test One, the number secretly selected by the subject was detected from the GSR in 80% of the controls and 65% of the patients. This test served chiefly as an indicator of the effectiveness of the test circuit and of the type of reaction which the subject gave. On examining the records later, the ease of detection of the number was listed as either "very clear," "clear," "fair," or "missed." These reactions were sometimes used later in comparing the types of GSR shown by the subject to the pain stimulus and to the conditioning stimulus. It is of interest that there was less consistency in the responses of the patients than in those of the controls, since this variability appeared in some of the later tests of the patients also, but in spite of other attempts at correlation with type of clinical diagnosis or state of anxiety, nothing more objective emerged from this procedure.

In Test Two the thresholds for touch and pain as detected subjectively were noted. As can be seen from Table I, threshold for the perception of touch varied between 10 and 50 volts. There were eight subjects on whom touch thresholds were not recorded as it was not recognized in these earlier tests that it might be significant. There were 18 (14 males) of the remaining 51 controls and eight (two males) of the 22 patients with touch thresholds above 20.

TABLE II

Mean thresholds for touch (T), pain (P), galvanic skin response (GSR) and conditioning times (CR)

	Mean thresholds			
	T	P	GSR	CR
Patients, male	25.00	71.25	62.00	10.42
Patients, female	22.86	60.63	57.50	7.64
Controls, male (pain)				10.38
Controls, female (pain)				8.33
Controls, male (sound)				13.50
Controls, female (sound)				8.00
Total controls, male	28.33	75.17	66.33	11.94
Total controls, female	18.62	63.33	56.82	8.47

Pain threshold varied between 30 and 90 volts in the controls and between 30 and 90 volts in the patients. Table II shows the means for the two groups and for the sexes. It will be seen that the thresholds for touch and pain are higher in the males than in the females in both patients and control group (Figure 1). But when the 24 patients were matched for age and sex with 24 of the controls (Figure 2), no difference between the two became apparent.

The results of Test Three, conditioning to pain and sound, are shown in Figure 3. With either stimulus there was no significant difference be-

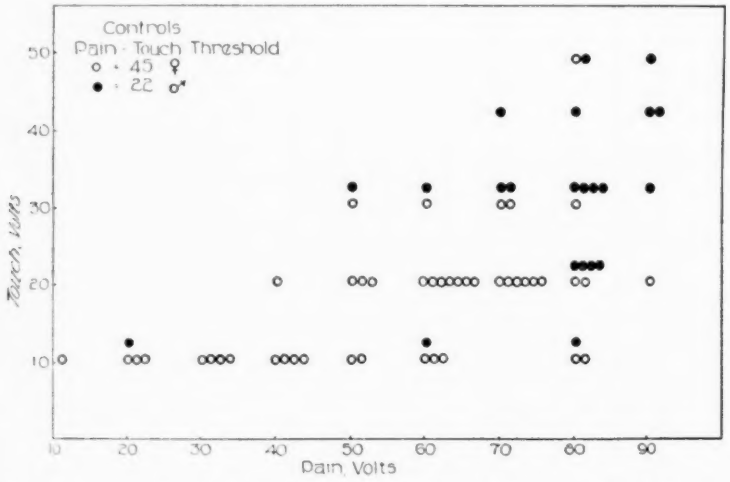

FIG. 1. PAIN AND TOUCH THRESHOLD OF CONTROL GROUPS SHOWING A HIGHER THRESHOLD FOR MALES THAN FOR FEMALES

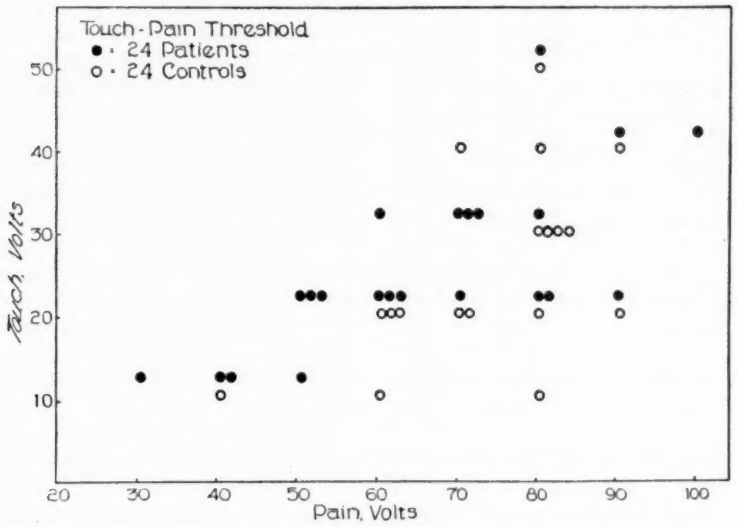

FIG. 2. PAIN THRESHOLDS FOR 24 PATIENTS AND 24 CONTROLS SHOWING NO SIGNIFICANT DIFFERENCES

Here, the age and sex of the 24 patients has been matched by that of the selected controls.

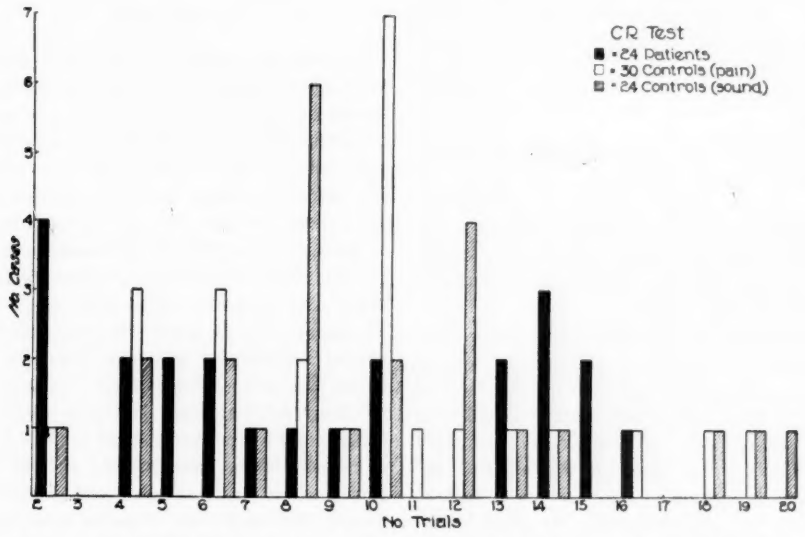

FIG. 3. CONDITIONING TIME AND NUMBER OF TRIALS OF 24 PATIENTS AND 30 CONTROLS USING PAIN AS THE CONDITIONING STIMULUS AND FOR 24 CONTROLS USING A SOUND STIMULUS

No significant differences appear.

tween controls and patients. When the controls, using the pain stimulus as originally planned, showed a much lower level for conditioning rate than had been previously reported as normal (4), it was thought that this might be due to the fact that a pain stimulus was more anxiety-inducing than a sound stimulus. A second control group was therefore selected, using sound as in the original procedure, instead of pain (4). There was no difference in conditioning time between the three groups, although a slight sex difference in all three appeared. Since the conditioning time varied only between two and 26, and since there were only seven of the 81 subjects who conditioned in more than 15 trials, differences would have had to be very large and clear-cut to be significant. These did not appear.

Welch and Kubis divided their patients according to whether they conditioned below six trials or above 14, and found positive correlation between such groupings and the clinically rated amount of anxiety. When the 24 patients of our group were classified according to their clinical anxiety as described above, eight patients were thought by all observers to be "most anxious" and eight "least anxious." The average conditioning time of the "anxious" group was 6.9 and that of the less anxious 13.0. Both these means, however, are below the average for normal as designated by Welch and Kubis.

Three patients and two controls gave results which could not be interpreted. Of these, two patients and one control reacted so strongly to every syllable that no conditioning could be measured. The other two never developed a conditioned reaction although responses to unconditioned stimuli were present.

The thresholds for the GSR as recorded in Test Four showed no significant difference between the controls and patients although the responses of the patients were more widely scattered than were those of the controls. Although pain threshold for males was higher than for females, this difference was less great in the *reaction to pain* as indicated by the GSR threshold. As might be expected by the data of Furer and Hardy (3), the GSR was much more variable than was the pain threshold. Seven of the 24 patients, but none of the controls, had reactions which were so confused that no threshold could be determined. Five of these had no GSR to any stimulus given during the fourth test although they had shown definite reactions to smaller stimuli during the number and conditioning tests. They must, therefore, have accommodated to the GSR during the previous testing. The other two had such marked over-reactions both during the GSR and the conditioning tests that no threshold or conditioning time could be determined. No correlation was found between these unusual reactions and anxiety; presence or absence of pain; or clinical diagnosis. But the conditioning time of the seven whose GSRs were undeterminable in Test Four averaged 6.8 which is relatively fast as compared to the average for all patients.

There was also slightly more variability or irregularity in pain GSR relationships of the patients than of the controls. In the controls the usual GSR threshold was either equal or slightly below the pain threshold, but this relationship was much more variable among the patients. Similarly, extraneous GSR responses appeared more often and were more marked in the patients' records.

DISCUSSION

The original purpose of this investigation was to discover whether there were any significant differences between the responses to painful stimuli of patients who had endured long and severe pain and controls who had not. Most of the findings are negative. There is not, as shown by these tests chosen to measure various qualities related to pain, any significant difference in threshold, reaction time or conditioning to pain, between patients and controls. The data are reasonably consistent and, therefore, assumed valid. They expose a number of interesting points in relation to our present knowledge of the physiology of pain.

Thresholds for touch and pain were detected consistently by the subjects and it was felt that the electrical stimulus used was the one of choice for this purpose both because the procedure was greatly shortened when compared to that necessary in using a heat stimulus and because the pain threshold so detected had been shown to be fairly consistent in previous tests repeated on the Laboratory staff.

In these tests the threshold for both touch and pain was higher in males than in females. There are several possible reasons for this, but no completely satisfactory explanation. It may be that, in the males, the skin of the forearm is thicker. The oldest and thickest-skinned males were compared, therefore, with the youngest, and probably most tender-skinned but no difference in threshold was apparent. Moreover, if the skin thickness were important, it should also have affected the pain threshold for heat radiation, but there is no such sex difference reported by those who have used the latter test. Another responsible factor might be the mores of the present time. Men are not supposed to cry out to pain as quickly and easily as are women. Whether this could influence our data is difficult to decide, and again, if these habits were responsible they might have affected the data of Wolff and his associates also. However, their tests were carried out largely on highly trained and sophisticated controls which might have influenced this special point. Furthermore, the fact that our GSR levels for the two sexes were more nearly alike than were touch or pain thresholds, might also indicate habit differences, for the GSR is less "voluntary" than is the subjective response to stimuli.

The GSR, like the pain threshold, produced nothing of interest which might differentiate patient from control except that, in general, reactions were more variable and less clearly defined in the patient group. Nothing could be found which related clinical features such as anxiety or a functional overlay, to any type of reaction. The heightened variability in the patients' test may be due to one of several factors. These patients, having only their pain in common were a more heterogeneous lot than were the controls, since the patients had greater range in age and since their educational and economic backgrounds were more varied. In a number of cases it was felt by the observers that the attention of the patients was less well focused than that of the control group. This was, in some instances, due to the fact that the patient was relatively uncomfortable because of his existent pain. In others, the greater drive and interest of the patient in the tests appeared to make his attention wander away from the particular focus—to the observer, the noise of the machine, etc. Anxiety, in this immediate sense, as set up in relation to the tests was thought to be a deterrent from efficient performance rather than a stimulus to it.

The fact that both control groups had mean levels for conditioning time which were about equal to that of the patients is not necessarily surprising, but the fact that the control groups showed levels which would place them, according to the categories of Welch and Kubis (4), in a group having high anxiety status is difficult to explain and, in itself, invalidates any conclusions which might be drawn from the low conditioning time of the patients. No explanation has been found for this difference between our controls and the normals previously reported. Since the author had previously worked with Dr. Welch, it is probable that the experimental set-up in the present investigation was as nearly like his as possible. As there was no difference between conditioning time of those responding to pain stimuli and to sound, the unconditioned stimulus could not be responsible. It was originally thought that the subjects selected were as nearly like those of Dr. Welch as possible, but it is conceivable that the tension and anxiety factor in a group of graduate students in nursing or medical schools is higher in test situations than that of the more sophisticated psychology classes of Hunter College. The processes underlying this difference are not easy to detect. The fact that the project had to do with *pain* may have had some influence. The personality and performance of the observer may also have been effective. All that is shown, apparently, by the test is that the patient and control groups are similar in their performances, and that there are other factors as well as those of anxiety, originally described as relevant, which have not yet been evaluated and which must play a part in the interpretation of the test.

In concluding, it should be noted that the data obtained from psychological tests on these same patients, which are to be published elsewhere, showed similar findings to the above in that the mental status and personality types of the patients showed no uniformity of trend which could be related either to functional or anxiety factors or to the behavioral reactions to pain. This group of 24 patients, in spite of the fact that most of them

had previously been categorized as being over-reactive or having functional elements to their pain reacted on the whole like any other group of individuals except that in addition to their many and varied and normal characteristics, they had also their severe and chronic pain.

CONCLUSIONS

1. Comparisons have been made between the responses to painful stimuli of normal control subjects and of patients having chronic clinical conditions involving severe, long-standing pain.

2. The thresholds for touch and pain perception were recorded. No significant difference appeared between the levels of the controls and those of the patients.

3. Reaction to pain, as indicated by the galvanic skin response, was also similar in the control and in the patient groups. As was to be expected from the work of previous authors, reaction to pain was more variable and less constant than was pain threshold.

4. Conditioning to painful and to auditory stimuli proved to be alike in the two groups.

5. Significant differences in touch and pain threshold and slight differences in GSR threshold and in conditioning time, appeared between males and females irrespective of whether they were in the control or patient groups.

6. In spite of the fact that all patients had signs of focal organic pain-inducing lesions, about 80% had been previously diagnosed as "functional." But there was no evidence that anxiety and tension, as measured by the present tests, differed in the patient and control groups.

7. The only difference observed in test results was that the responses of the patients were more varied and less clear-cut than those of the controls. This variability could not be related to any known factor within the groups unless it was that the attention of the patients was less well directed and maintained.

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DIURNAL VARIATION IN THE URINARY EXCRETION OF NEUTRAL LIPID-SOLUBLE REDUCING STEROIDS IN CONGESTIVE CARDIAC FAILURE AND CIRRHOSIS OF THE LIVER WITH ASCITES [1, 2]

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Pincus and his collaborators (1–4) have demonstrated that there is a normal cycle of excretion of neutral, lipid-soluble reducing steroids which has a maximum between 7 a.m. and 11 a.m., decreases during the afternoon, and is at a minimum during the night. This corresponds to the normal excretory cycle of other urinary constituents (5–13). It has also been shown that in congestive cardiac failure (14, 15) and in cirrhosis of the liver with ascites (16) there is a reversed diurnal cycle of water and sodium excretion, with maximum values during the night in a high proportion of the individuals studied. This investigation was designed to determine if the excretion of steroids was also altered by these conditions.

MATERIAL AND METHODS

Ten normal male adults were used as controls. Fourteen male patients with congestive heart failure, of various etiologies and in differing degrees of decompensation, and 12 male patients with cirrhosis of the liver and ascites comprised the abnormal groups. Almost all of the patients were on a standard hospital diet containing a calculated 1,000 milligrams of sodium daily. Diuretics were not used within 48 hours of the urine collection periods, and no clinical evidence of urinary obstruction was present. Three consecutive, eight-hour, spontaneously voided urine specimens were collected from each individual. The one collected between 6 a.m. and 2 p.m. was designated the morning specimen, the one collected between 2 p.m. and 10 p.m. was designated the afternoon specimen, and the one collected between 10 p.m. and 6 a.m. was designated the night specimen. The time of the night specimen was chosen so that it would coincide most closely with the hours of sleep. The volume of each urine was measured and the quantity of neutral

[1] Reviewed by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions of the authors are the results of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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TABLE I

Normal subjects

Pt.	Age	Date	Steroids*				Water			
			Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night
			mg.	%	%	%	*ml.*	%	%	%
C. D.	30	Feb. '51	2.95	52	22	26	1,165	54	14	32
W. A.	31	Feb. '51	3.25	45	25	30	1,550	61	16	23
S. B.	54	Mar. '51	3.23	38	37	25	1,190	45	34	21
E. M.	30	Mar. '51	3.43	45	29	26	1,070	65	20	15
W. M.	30	Mar. '51	2.62	39	30	31	1,425	50	19	31
R. G.	31	Sept. '50	2.51	40	29	31	1,822	37	30	33
W. F.	29	Sept. '50	2.60	50	30	20	1,230	54	32	14
G. M.	60	Apr. '51	2.63	38	33	29	1,065	40	30	30
M. L.	30	Mar. '51	4.35	37	33	30	1,370	33	31	36
W. B.	30	May '51	2.47	35	35	30	1,153	40	30	30
Standard deviation			0.59	5.8	4.5	3.5	240	10.6	7.5	7.7
Mean			3.00	**42**	30	28	1,304	**48**	26	26

* Neutral lipid-soluble reducing steroids.
Boldface value for mean indicates that difference from mean night value exceeds 0.05 level of confidence.

TABLE II

Congestive cardiac failure

Pt.	Age	Date	Steroids*				Water				Sodium				Creatinine				Remarks
			Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	
			mg.	%	%	%	*ml.*	%	%	%	*mg.*	%	%	%	*mg.*	%	%	%	
E. P.	58	Sept. '50	2.28	41	27	32	1,034	31	41	28	730	36	29	35	1,190	33	34	33	Old myocardial infarction. Compensated.
E. P.	58	May '51	3.15	25	34	41	1,390	24	40	36	524	13	8	79	795	26	41	33	Decompensated. Died June '51 of new infarct.
E. J.	62	Sept. '50	3.51	48	27	25	1,225	21	39	40	524	16	28	56	1,126	30	32	38	Hypertensive and arteriosclerotic heart disease. Old myocardial infarction. Aortic aneurysm. Compensated.
W. K.	57	Mar. '51	3.95	30	38	32	2,530	24	23	53	2,213	29	3	68	1,392	34	36	30	Arteriosclerotic heart disease. Old myocardial infarction. Decompensated. Improving.
J. T.	51	Mar. '51	3.95	27	32	41	1,495	23	19	58	19	21	26	53	1,063	29	33	38	Rheumatic heart disease. Mitral stenosis, severely and recurrently decompensated. Improving. Died suddenly July '51 of pulmonary embolism.
E. L.	73	Mar. '51	1.79	18	41	41	1,036	12	33	55	1,350	10	35	55	808	16	39	45	Hypertensive and arteriosclerotic heart disease. Decompensated. Improving.
G. W.	32	Apr. '51	3.41	38	31	31	925	35	31	34									Mitral and aortic valvulitis with healed subacute bacterial endocarditis. Severely decompensated, pulmonary emboli. Died April '51.
E. D.	28	Apr. '51	3.59	29	32	39	1,515	32	38	30									Rheumatic heart disease, aortic and mitral valvulitis, subacute bacterial endocarditis. Moderately and recurrently decompensated. Improving.
J. H.	61	Apr. '51	2.24	27	44	29	1,375	21	46	33									Rheumatic heart disease, mitral stenosis and insufficiency, auricular fibrillation, recurrently decompensated. Improving.
C. T.		Apr. '51	1.41	30	31	39	995	30	23	47	1,363	32	17	51	830	32	25	43	Arteriosclerotic heart disease, decompensated, improving.
C. W.	58	May '51	3.51	39	39	22	1,445	28	52	20	1,289	25	52	23	1,106	39	41	20	Pulmonary fibrosis and emphysema, cor pulmonale, chronic congestive failure, improving.
J. C.	63	May '51	2.43	28	28	44	625	23	33	44	214	17	41	42	1,407	25	35	40	Hypertensive and arteriosclerotic heart disease. Decompensated, improving.
A. Z.	50	May '51	3.11	30	28	42	1,295	22	33	45	1,704	18	37	45	2,260	26	30	44	Rheumatic heart disease with mitral stenosis. First time decompensated. Improving.
H. P.	57	May '51	3.21	28	32	40	1,220	23	32	45	815	16	30	54	1,449	23	37	40	Luetic heart disease. Mildly decompensated. Improving.
J. P.	57	Jun. '51	2.80	30	46	24	1,280	27	43	30	223	25	45	30	1,517	27	48	25	Arteriosclerotic heart disease. Old myocardial infarction. Decompensated. Improving. Died suddenly a few days after study.
Standard deviation			0.79	7.4	6.2	7.3	420	5.6	9.0	11.0	†	8.0	14.5	15.6	408	6.1	6.0	7.8	
Mean			2.96	31!	34	35!	1,292	25!	35!	40!	914	**22**	**29**	49	1,245	**28**	36	36	

* Neutral lipid-soluble reducing steroids.

† Standard deviation not determined; several patients not on 1,000 milligram sodium diet.

Boldface value for mean indicates that difference from mean night value exceeds 0.05 level of confidence.

Exclamation point after mean value indicates that difference from corresponding normal value exceeds 0.05 level of confidence.

TABLE III

Cirrhosis of the liver with ascites

Pt.	Age	Date	Steroids*				Water				Sodium				Creatinine				Remarks
			Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	
			mg.	%	%	%	*ml.*	%	%	%	*mg.*	%	%	%	*mg.*	%	%	%	
E. L.	61	Jul. '50	2.39	23	43	34	891	10	60	30	340	1	31	68	1,529	30	35	35	Moderately severe ascites, improving, ambulatory.
O. R.	55	Jul. '50	1.61	23	48	29	1,138	17	63	20	164	18	35	47	1,372	30	34	36	Moderate, increasing ascites, ambulatory.
O. L.	52	Oct. '50	2.07	36	26	38	723	23	38	39	457	20	66	14	1,155	28	28	44	Severe, increasing ascites, ambulatory.
A. R.	55	Oct. '50	2.87	34	37	29	1,120	26	36	38	400	26	16	58	1,231	28	40	32	Severe ascites, ambulatory.
R. M.	58	Aug. '50	2.12	12	36	52	1,900	45	21	34	30	9	30	61					Moderate ascites, ambulatory. Died Sept. '50 of hemorrhage.
H. G.	57	Mar. '51	4.56	22	29	49	1,575	15	39	46	123	6	8	86	1,298	23	35	42	Severe, progressive ascites, ambulatory. Died May '51 of hemorrhage.
E. H.	60	May '51	1.79	26	20	54	172	29	19	52	15	30	15	55	278	31	19	50	Severe ascites, absolute bed rest.
J. V.	41	May '51	6.40	29	37	34	1,320	19	47	34	2,821	20	54	26	1,045	26	38	36	Ascites cleared, ambulatory.
W. L.	47	May '51	4.35	48	27	25	935	70	16	14	154	45	26	29	957	42	33	25	Severe ascites, absolute bed rest.
A. H.	54	Jun. '51	3.88	30	39	31	1,080	20	28	52	680	11	31	58	1,335	27	36	37	Decreasing ascites, slightly ambulatory.
S. H.	41	Jun. '51	3.99	43	32	25	1,100	41	37	22	69	38	38	24	1,231	41	35	24	Severe ascites, lavatory privileges only. Died July '51 of peritonitis.
H. H.	43	Jun. '51	3.83	35	22	43	1,140	25	12	63	349	15	15	70	979	33	16	51	Ascites subsiding. Ambulatory.
Standard deviation			1.40	9.9	8.4	10.3	425	16.6	16.4	14.6	†	13.0	16.8	21.9	331	5.9	7.7	8.9	
Mean			3.32	31!	33	37!	1,091	28!	35	39!	467	**20**	**30**	50	1,128	31	32	37	

*Neutral lipid-soluble reducing steroids.
† Standard deviation not determined; several patients not on 1,000 milligram sodium diet.
Boldface value for mean indicates that difference from mean night value exceeds 0.05 level of confidence.
Exclamation point after mean value indicates that difference from corresponding normal value exceeds 0.05 level of confidence.

lipid-soluble reducing steroids [a] determined by the method of Heard, Sobel and Venning (17). In almost all of the specimens obtained from the cardiac and cirrhotic patients, the quantity of excreted sodium was determined by means of the Beckman flame photometer and the quantity of excreted creatinine was determined by the Bonsnes and Taussky (18) modification of the Jaffe reaction.

RESULTS

The tables summarize the clinical data and the results. The total amount of the excreted substance is indicated in the first column of each group, and the succeeding three columns indicate the amount excreted in the morning, afternoon and night periods, respectively, expressed as per cent of the total amount excreted.

Table I indicates the results obtained in the normal subjects. All demonstrated a morning maximum steroid excretion, and most had a morning maximum water excretion. The average 24-hour excretion of steroids was 3.00 milligrams.

The pattern of excretion of steroids, water, sodium and creatinine in 14 patients with congestive cardiac failure is seen in Table II. The average 24-hour excretion of steroids was 2.96 milligrams, which was remarkably close to the normal value. However, the distribution of the excreted steroids showed a definite alteration: in only four studies was the maximum excretion in the morning, and in two of these, the patients (E. P. and E. J.) were very well compensated by intensive therapy. The steroid pattern resembled the excretory cycles of the water, sodium and creatinine. It can be seen that the fluctuations in excretion of steroids, water and creatinine from period to period are of the same order, but that excretion of sodium shows more marked variations.

The observations made on 12 patients with cirrhosis of the liver and ascites are seen in Table III. The results compare closely to those of the cardiac group. The average 24-hour excretion of steroids, 3.32 milligrams, is slightly higher than for the other two groups. However, these differences are not statistically significant. It can be seen that the excretory cycle is abnormal in most instances, that the fluctuation in the water, creatinine and steroid cycles is less than normal, while fluctuation in the sodium cycle is proportionately greater, and that most of the variations in excretion of the observed substances from period to period are in the same direction. Despite the apparent trend, the differences in the creatinine values are not significant, and cannot be interpreted to demonstrate a nocturnal increase in the glomerular filtration rate.

[a] Hereafter referred to as steroids.

DISCUSSION

Most studies on diurnal variation in urinary excretion have concerned the normal patterns. These patterns have been well documented, and there is general agreement among investigators that the maximum output of water, sodium, potassium, chloride, urea and creatinine is during the day, with a peak either just before or just after noon (5–13). Recently, a number of studies on abnormal variations have appeared, especially pertaining to the nocturnal diuresis found in patients with congestive cardiac failure (14, 15). We have recently shown that there is a similar nocturnal diuresis in cirrhosis of the liver with ascites (16). Although a nocturnal increase in renal plasma flow has been demonstrated in congestive cardiac failure (14, 15), which is associated with an increased glomerular filtration rate, this does not necessarily explain the entire mechanism of the nocturia. To our knowledge no similar studies of renal plasma flow and glomerular filtration rate have been performed on patients with cirrhosis of the liver. In both of these conditions a nocturnal diuresis of water and sodium has been observed, but in our series (16) most of the patients exhibited no consistent associated diuresis of potassium.

The work of Pincus and his collaborators demonstrated that normally there was a maximum excretion of steroids during the morning, a decreased excretion during the rest of the day, and a minimum during the night. They believed that the morning increase was not due to increased filtration alone, since the excretion of steroids showed a greater proportionate increase than did the excretion of creatinine. The variation was considered to be the result of the increased stress due to resumption of activity after sleep. They noted that specific stress situations were associated with an increase in adrenal cortical steroid excretion occurring within a short time following the application of the stress.

The present studies confirm the nature of the steroid excretory cycle in normal individuals. However, there is a fairly consistent relationship between the excretion of steroids, water and creatinine, suggesting that there may be a similar excretory mechanism for each, and that under these conditions excretion may be related to the filtration rate. A similar relationship is seen in congestive failure and cirrhosis, although there is displacement in the time of peak excretion. This shift in the cycle cannot be attributed to periods of excessive stress, first because the total steroid excretion is no greater than in the normals, and second because there is no evidence of an altered stress cycle. It may be suggested that in some of the patients with congestive cardiac failure nocturnal episodes of paroxysmal dyspnea and insomnia may be related to increased stress; however, patients who slept soundly during the collection period also demonstrated increased nocturnal steroid excretion. None of the patients with cirrhosis showed evidence of disturbed sleep other than that caused by the inconvenience of the nocturia itself. It is apparent either that more steroids are being presented to the kidney during the night, or that the kidney is relatively more efficient in excreting the steroids at that time.

From the data it appears that the amount of steroid excreted in 24 hours is relatively constant, and that the values for patients with congestive cardiac failure and cirrhosis of the liver are not significantly different from the normals. Abnormal patterns of diurnal urinary excretion of water, sodium, and creatinine are associated with similarly abnormal patterns of steroid excretion. It does not seem possible to determine from the data whether this abnormal excretory pattern for water, sodium and creatinine results from the pattern of steroid excretion, or whether the rate of steroid excretion is itself governed by the same alternate factors. In addition to the absence of evidence of increased adrenocortical activity, there was no evidence of an alteration in the stress cycle of these patients. Gaunt, Birnie and Eversole (19) have reviewed data demonstrating that an adequate amount of cortical hormone is necessary for water diuresis. However, they point out that at the same time as there is increased cortico-steroid there is usually an increased retention of sodium. While this may be true on an overall balance, fluctuations in sodium excretion during the day are in the same direction as fluctuations in steroid excretion, rather than in the opposite direction as would be expected.

CONCLUSIONS

1. The average 24-hour excretion of neutral lipid-soluble reducing steroids was 3.00 milligrams in ten normal adults, 2.96 milligrams in 14 patients with decompensated or recompensated cardiac failure, and 3.32 milligrams in 12 patients with cirrhosis of the liver and ascites.

2. In all of the normal controls there was a significant morning maximum excretion of steroids. In both of the abnormal conditions studied the period of maximum excretion was most commonly observed in the afternoon or during the night.

3. With few exceptions, the excretion of steroids in cardiac failure and cirrhosis was proportional to the excretion of creatinine within the limits of error of the methods. This suggests that the excretion of steroids may be proportional to the glomerular filtration rate.

4. Since the total steroid excretion is within normal range, there is no evidence of increased or abnormal stress as a cause of the abnormal steroid excretory pattern. It is possible that the glomerular filtration rate is proportional to adrenocortical activity as measured by steroid excretion. However, it seems more probable that the urinary content of the steroid is proportional to the excretory rate.

5. In the normal and abnormal states studied, the water excretion generally paralleled that of creatinine and steroids, while the sodium excretion showed more marked variations, usually, but not invariably, in the same direction.

ACKNOWLEDGMENT

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THE EFFECTS OF POTENT ANALGESICS UPON VESTIBULAR FUNCTION

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With the introduction of potent synthetic analgesics, it became evident upon clinical evaluation that the ambulatory patient more frequently displayed the signs and symptoms of toxicity than the bed-ridden patient. Thus, the occurrence of dizziness, nausea and vomiting was appreciably increased in the patient who was up and about and who was receiving meperidine and methadon (1–4). This phenomenon was not readily appreciated in the case of morphine in ambulant patients because this drug is used principally for seriously-ill bed patients and because of its decreased effectiveness when administered by the oral route. However, it had been recognized by many studying the effects of morphine in man that untoward reactions may occur more frequently in the upright position (5). Two possible approaches to the investigation of this problem were considered, the influence of such medications upon cardiovascular dynamics and their possible alterations of vestibular function. Since preliminary investigations in our laboratory failed to reveal any significant aberrations in cardiovascular dynamics with these drugs (6) this study was initiated to determine the effects of potent analgesics upon labyrinthine function.

METHOD

The methods employed were the cold micro-caloric test (7, 8) and galvanic stimulation of the mastoid area (9). The former consists of the injection of 2 cc. of ice water (water with floating ice) into the external auditory canal with the head inclined laterally so that the ear to be tested is uppermost. The same ear is used throughout the test. The time for the onset of nystagmus to the contralateral side is recorded with a stop-watch; and, at that point, the head is returned to the upright position, and the canal emptied of water. The duration of the true nystagmus is then noted. This test measures the sensitivity and responsiveness of the peripheral end-organ in the labyrinth and lends itself to close repetition because of its simplicity and lack of unpleasantness to the subject. The ratio of the onset to the duration of nystagmus is used as a measure of labyrinthine sensitivity for comparative purposes, a decrease in this ratio being indicative of an increased responsiveness and an increase having the opposite implication.

The galvanic stimulation test is performed with the subject standing balanced on a tilt board holding the moistened cathode in one hand while the examiner applies the moistened anode to the contra-lateral mastoid area. The milliamperage necessary to effect tilting is noted. This method depends upon the integrity and sensitivity of the vestibular nerve tract and associated brain centers; and it, too, is devoid of complexity and rejection by the patient.

Sixteen ambulatory patients, none of whom presented any abnormalities of the ear, underwent 25 complete trials with one or more of the following drugs: codeine sulfate 60 mgms., meperidine hydrochloride 50 mgms., morphine sulfate 10 mgms., methadon 10 mgms., pantopon 20 mgms., and acetylsalicylic acid 0.6 gm. All medications with the exception of acetylsalicylic acid were given intramuscularly. In four additional subjects, six complete observations were accomplished in which dimenhydrinate 100 mgms. was ingested orally, followed 15 minutes later by the intramuscular administration of meperidine hydrochloride 50 mgms. or morphine sulfate 10 mgms. in three instances each.

The following was the procedure employed. The control period consisted of three micro-caloric determinations, at approximately five minute intervals, followed by a single base-line determination of the milliamperage required for tilting. One of the above drugs or combination of drugs was then administered. At roughly 20 minute intervals one micro-caloric test and one galvanic stimulation were done, for a total of at least three such determinations or until it was felt that the maximum vestibular effects had been produced and were receding.

RESULTS

The effects of meperidine hydrochloride, morphine sulfate, methadon hydrochloride and codeine sulfate upon the onset and duration of nystagmus and their ratios are presented in Tables I–IV and Figures 1–3. For comparative purposes five representative cases from our previously reported experiences (10) with dimenhydrinate (dramamine) are included in these figures.

At this point several explanations are necessary for the better understanding of the ensuing tables.

TABLE I

Influence of meperidine hydrochloride (50 mgms. administered intramuscularly) on vestibular function, as measured by the cold micro-caloric method

Subject	Age (yrs.)	Time of onset of nystagmus in seconds				Per cent of change	Duration of nystagmus in seconds				Per cent of change	Ratio of time of onset and duration of nystagmus	
		Control*	Meperidine				Control*	Meperidine				Control	Meperidine†
			1	2	3			1	2	3			
L. B.	55	27 (30, 31, 21)	**29**	32	—	0	90 (98, 90, 81)	**129**	107	—	+43	1:3.3	1:4.4
M. M.	47	22 (23, 22, 21)	**22**	20	22	0	93 (92, 89, 98)	**143**	80	86	+54	1:4.2	1:6.5
A. H.	77	25 (27, 26, 23)	26	**22**	20	−20	84 (89, 84, 79)	129	**131**	108	+56	1:3.4	1:6.0
W. M.	45	31 (27, 33, 34)	39	**26**	25	+24	88 (83, 88, 93)	98	**128**	87	+45	1:2.8	1:5.0
M. K.	39	25 (24, 22, 28)	23	**21**	28	0	96 (90, 99, 99)	134	**149**	102	+55	1:3.8	1:7.1

* Average of three determinations.
† Maximal effect of drug.
Note: Boldface numbers are components of ratios in last column on right.

TABLE II

Influence of morphine sulfate (10 mgms. administered intramuscularly) on vestibular function, as measured by the cold micro-caloric method

Subject	Age (yrs.)	Time of onset of nystagmus in seconds				Per cent of change	Duration of nystagmus in seconds				Per cent of change	Ratio of time of onset and duration of nystagmus	
		Control*	Morphine sulfate				Control*	Morphine sulfate				Control	Morphine sulfate†
			1	2	3			1	2	3			
M. K.	39	24 (23, 22, 27)	22	**24**	25	0	111 (111, 110, 112)	115	**147**	114	+32	1:4.6	1:6.1
M. N.	58	29 (28, 27, 31)	32	**20**	22	−31	85 (82, 90, 84)	106	**120**	91	+41	1:3.0	1:6.0
F. W.	48	28 (24, 33, 26)	19	**18**	28	−36	113 (107, 121, 110)	131	**130**	120	+16	1:4.0	1:7.2
L. B.	55	27 (27, 26, 27)	36	**22**	26	+33, −18	88 (96, 74, 95)	115	**114**	82	+31	1:3.3	1:5.2
M. S.	60	22 (20, 22, 23)	18	**18**	20	−18	90 (87, 90, 92)	110	**130**	118	+44	1:4.1	1:7.2

* Average of three determinations.
† Maximal effect of drug.
Note: Boldface numbers are components of ratios in last column on right.

TABLE III

Influence of methadon (10 mgms. administered intramuscularly) on vestibular function, as measured by the cold micro-caloric method

Subject	Age (yrs.)	Time of onset of nystagmus in seconds				Per cent of change	Duration of nystagmus in seconds				Per cent of change	Ratio of time of onset and duration of nystagmus	
		Control*	Methadon				Control*	Methadon				Control	Methadon†
			1	2	3			1	2	3			
A. P.	22	31 (32, 30, 32)	28	**28**	30	−10	102 (102, 98, 106)	115	**120**	115	+18	1:3.3	1:4.3
L. K.	25	26 (25, 28, 26)	23	**24**	26	−11	97 (98, 94, 100)	110	**133**	108	+37	1:3.7	1:5.5
M. A.	21	27 (30, 25, 25)	**17**	19	17	−37	103 (113, 97, 98)	**133**	118	123	+29	1:3.8	1:7.8
M. N.	58	24 (26, 22, 25)	21	**17**	16	−33	92 (96, 90, 91)	122	**116**	92	+33	1:3.8	1:6.8
M. K.	39	24 (24, 29, 18)	**24**	24	21	0	102 (103, 100, 102)	**129**	118	105	+26	1:4.2	1:5.4

* Average of three determinations.
† Maximal effect of drug.
Note: Boldface numbers are components of ratios in last column on right.

TABLE IV

Influence of codeine sulfate (60 mgms. administered intramuscularly) on vestibular function, as measured by the cold micro-caloric method

Subject	Age (yrs.)	Time of onset of nystagmus in seconds				Per cent of change	Duration of nystagmus in seconds				Per cent of change	Ratio of time of onset and duration of nystagmus	
		Control*	Codeine				Control*	Codeine				Control	Codeine†
			1	2	3			1	2	3			
M. K.	39	25 (25, 25, 25)	22	**22**	28	−12 +12	106 (112, 100, 105)	77	**58**	89	−45	1:4.2	1:2.6
M. V.	65	30 (34, 25, 30)	**35**	30	32	0	113 (123, 101, 114)	**70**	103	104	−38	1:3.8	1:2
M. K.	78	24 (24, 24, 25)	**25**	24	27	+12	123 (128, 125, 116)	**113**	115	127	−8	1:5.1	1:4.5
W. M.	45	25 (21, 27, 26)	**24**	26	26	0	97 (103, 90, 98)	**77**	97	96	−21	1:3.9	1:3.2
M. M.	47	25 (29, 24, 23)	**24**	22	22	0	98 (97, 98, 99)	**76**	85	99	−22	1:3.9	1:3.1
L. B.	55	28 (27, 28, 30)	21	**27**	26	−25	99 (101, 101, 94)	86	**60**	76	−40	1:3.5	1:2.2

* Average of three determinations.
† Maximal effect of drug.
Note: Boldface numbers are components of ratios in last column on right.

All the original control data for the onsets and durations of nystagmus and their averages are recorded. The testing periods after the administration of the various drugs are designated by the numbers 1, 2, and 3 and represent approximately 20 minute intervals. The percentages of change are derived by comparing the maximal effect of a drug with the average control figure. Arbitrarily, any resultant quantity falling within the range of the control values or differing by only one second has been recorded as unchanged. The control ratios are calculated from the average control values. The ratios following drug administration are computed from quantities for the onsets and durations which reflect the maximal effects of these drugs (in boldface for easier interpretation) and which must of necessity have occurred during the same test interval.

Meperidine, morphine and methadon produced an increase in labyrinthine responsiveness. The

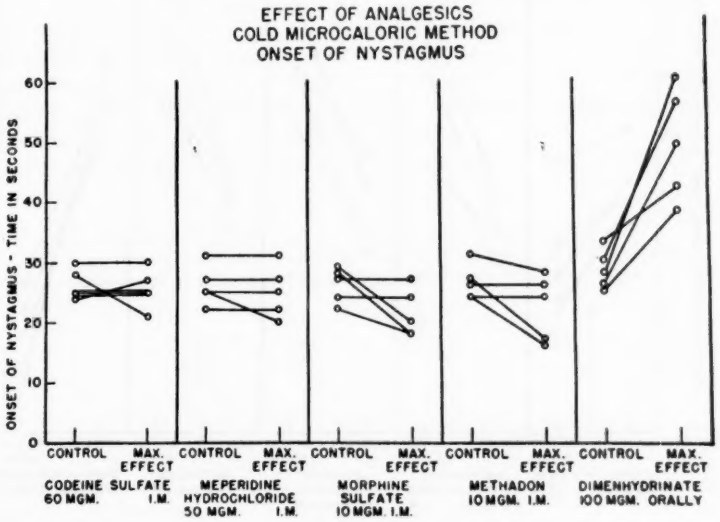

FIG. 1

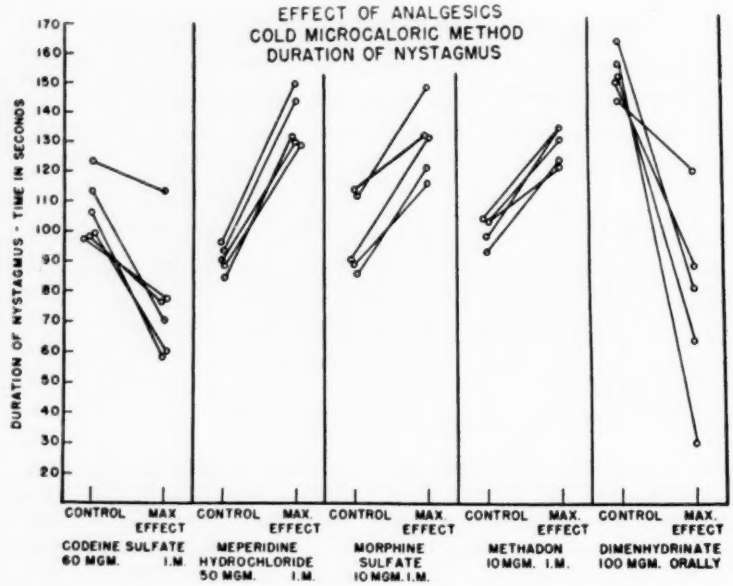
EFFECT OF ANALGESICS
COLD MICROCALORIC METHOD
DURATION OF NYSTAGMUS
DURATION OF NYSTAGMUS - TIME IN SECONDS
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
CONTROL
MAX. EFFECT
CODEINE SULFATE 60 MGM. I.M.
MEPERIDINE HYDROCHLORIDE 50 MGM. I.M.
MORPHINE SULFATE 10 MGM. I.M.
METHADON 10 MGM. I.M.
DIMENHYDRINATE 100 MGM. ORALLY

FIG. 2

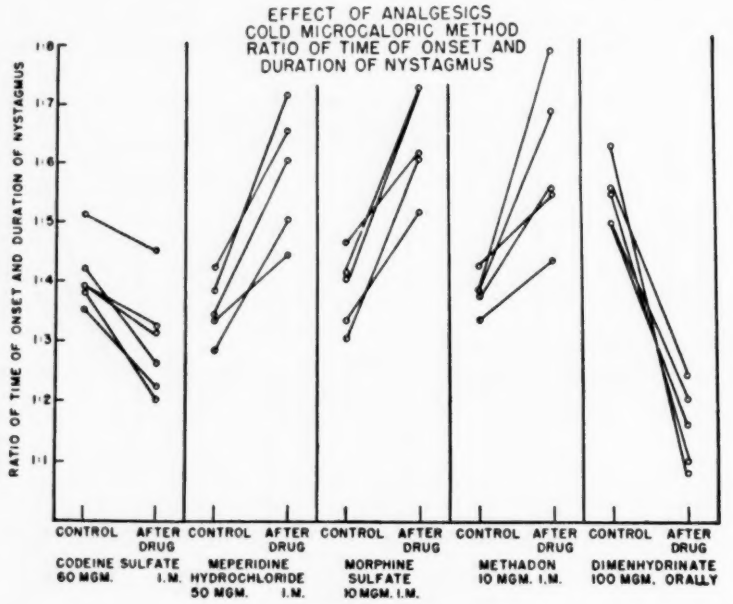
EFFECT OF ANALGESICS
COLD MICROCALORIC METHOD
RATIO OF TIME OF ONSET AND DURATION OF NYSTAGMUS
RATIO OF TIME OF ONSET AND DURATION OF NYSTAGMUS
1:8
1:7
1:6
1:5
1:4
1:3
1:2
1:1
CONTROL
AFTER DRUG
CODEINE SULFATE 60 MGM. I.M.
MEPERIDINE HYDROCHLORIDE 50 MGM. I.M.
MORPHINE SULFATE 10 MGM. I.M.
METHADON 10 MGM. I.M.
DIMENHYDRINATE 100 MGM. ORALLY

FIG. 3

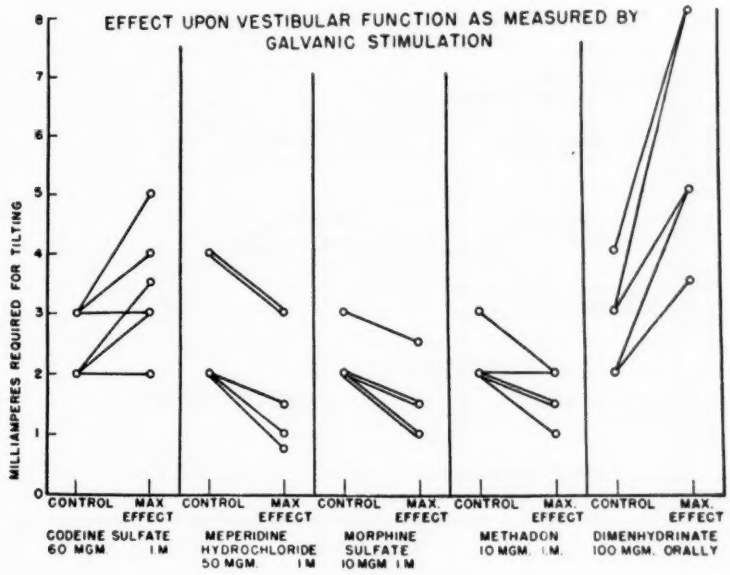

FIG. 4

onset of nystagmus was unaffected with meperidine, but both morphine and methadon decreased the onset of nystagmus in three out of five instances. In the five trials with each of these drugs, the duration of nystagmus was prolonged in every trial. The ratio of onset to duration was also decreased in every case.

The effects of these drugs upon vestibular function as measured by galvanic stimulation are presented in Figure 4. Again dimenhydrinate is included for comparison. With this method the amount of current necessary to effect tilting was decreased in every patient with these medications, except for one trial with methadon.

Codeine was the only drug of those included in this study which decreased the reactivity of the labyrinth. The onset of nystagmus was unchanged; however, the duration was decreased in every instance and the resultant ratio was materially raised in each of the six trials. Four of the six patients when tested galvanically exhibited an increase in the milliamperage needed to produce tilting.

Two patients were studied with pantopon and

TABLE V

Influence of pantopon (20 mgms. administered intramuscularly) on vestibular function, as measured by the cold micro-caloric method

Subject	Age (yrs.)	Time of onset of nystagmus in seconds				Per cent of change	Duration of nystagmus in seconds				Per cent of change	Ratio of time of onset and duration of nystagmus	
		Control*	Pantopon				Control*	Pantopon				Control	Pantopon†
			1	2	3			1	2	3			
M. M.	57	23 (24, 20, 24)	20	**18**	19	−9	91 (91, 88, 95)	125	**130**	88	+43	1:4.0	1:7.2
M. G.	48	19 (20, 19, 18)	**14**	18	18	−26	102 (97, 105, 105)	**130**	124	107	+27	1:5.4	1:9.3

* Average of three determinations.
† Maximal effect of drug.
Note: Boldface numbers are components of ratios in last column on right.

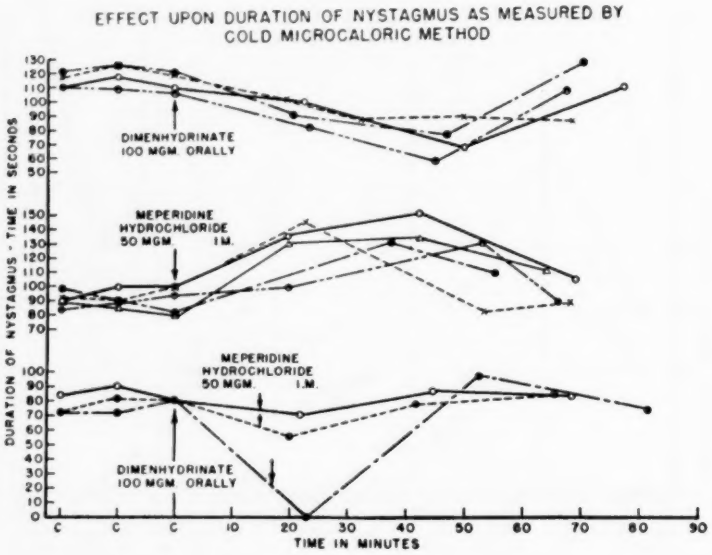
EFFECT UPON DURATION OF NYSTAGMUS AS MEASURED BY COLD MICROCALORIC METHOD
DURATION OF NYSTAGMUS - TIME IN SECONDS
DIMENHYDRINATE 100 MGM. ORALLY
MEPERIDINE HYDROCHLORIDE 50 MGM. I.M.
MEPERIDINE HYDROCHLORIDE 50 MGM. I.M.
DIMENHYDRINATE 100 MGM. ORALLY
C C C 10 20 30 40 50 60 70 80 90
TIME IN MINUTES

FIG. 5

the results were similar to morphine sulfate (Table V). Acetylsalicylic acid was administered orally in a dose of 0.6 gm. and had no effect upon vestibular function as measured by these methods.

Having thus established that meperidine, morphine and methadon stimulate the labyrinthine mechanism, it was felt from our previous studies on the pharmacology of dimenhydrinate that the use of this medication prior to the administration of these potent analgesics would offset their action upon the balance mechanism (10). This was borne out in two subjects in whom the vestibular effects of intramuscular meperidine were completely blocked by the use of 100 mgms. of dimenhydrinate orally. The third individual exhibited a partial inhibition of meperidine action. This is presented in Figure 5. The comparative effects of dimenhydrinate and meperidine hydrochloride when administered alone are shown in four and five subjects, respectively. The same results were observed in three trials with dimenhydrinate and morphine sulfate.

In addition to the above studies, the action of meperidine 50 mgms. intramuscularly was observed in three subjects in both the supine and upright positions on successive days with the aid of the cold micro-caloric method alone (Table VI). In all three cases the prolongation of the induced nystagmus was greater when the patient was ambulant than when he was lying in bed throughout the observation period. Because of this observation, the resultant ratios tended to reflect an overall increase in labyrinthine sensitivity in the upright position in spite of the fact that two patients in this position exhibited a prolongaton in the onset of nystagmus, a fact which ordinarily would tend to decrease the vestibular responsiveness. This again is in agreement with our previous clinical evaluation of these drugs, and probably results from the greater stress placed upon the balance mechanism in the upright position.

DISCUSSION

The effects of the potent analgesic drugs such as meperidine, morphine and methadon upon vestibular function indicate that the higher incidence of untoward reactions noted when these therapeutic agents are used in ambulatory patients may possibly be due to the resultant increased sensitivity of the labyrinthine apparatus. However, we feel that this is not the entire explanation since the administration of codeine which apparently depresses vestibular function, still is attended by this same increased frequency of untoward reactions

TABLE VI

Influence of meperidine hydrochloride (50 mgms. administered intramuscularly) on vestibular function, as measured by the cold micro-caloric method, in both supine and upright positions

Subject	Age	Time of onset of nystagmus in seconds								Duration of nystagmus in seconds								Ratio			
		Supine				Upright				Supine				Upright				Supine		Upright	
		Control*	Meperidine			Control*	Meperidine			Control*	Meperidine			Control*	Meperidine			Contr.	Mep.†	Contr.	Mep.†
			1	2	3		1	2	3		1	2	3		1	2	3				
D. M.	26	35 (30, 38, 37)	47	25	37	36 (35, 37, 36)	**33**	36	34	156 (170, 164, 133)	180	**200**	177	143 (133, 150, 145)	**225**	239	200	1:4.4	1:8	1:4	1:6.8
E. G.	47	31 (27, 30, 37)	28	29	31	31 (27, 33, 32)	35	**30**	32	159 (151, 163, 164)	157	**195**	171	144 (126, 145, 160)	154	**220**	178	1:5.1	1:6.7	1:4.6	1:7.3
H. V.	41	22 (15, 20, 30)	25	24	**28**	22 (19, 24, 23)	30	22	**18**	137 (130, 140, 140)	148	170	**181**	160 (158, 162, 159)	223	240	**285**	1:6.2	1:6.5	1:7.3	1:15.8

	% of chg.		% of chg.		% of chg.		% of chg.
D. M.	+34 −20	E. G.	0	H. V.	0	E. G.	+13
						H. V.	+36

* Average of three determinations.
† Maximal effect of drug.
Note: Boldface numbers are components of ratios in last column on right.

in ambulant patients. Other factors may also contribute to this phenomenon, such as stimulation of the cerebral emetic center, alterations in cerebral blood flow, and changes in cardiovascular dynamics not accurately measurable with available techniques.

Ross, Fish and Olsen in 1931 studied the effects of morphine in dogs by a rotational method, but were unable to demonstrate any increase in the duration of the induced nystagmus (11). In 1950 Rubin and Winston evaluated vestibular responses in man following morphine administration by means of the Barany chair and a macro-caloric method (12). They concluded that "morphine administration produced a significant decrease in vestibular response," yet they also concluded that the nauseant and emetic effects of morphine were markedly enhanced by vestibular stimulation. These conclusions appear to us to be mutually contradictory. Their findings of a decrease in vertigo and duration of postrotational nystagmus after morphine justify their conclusion of vestibular depression, but our studies with the cold micro-caloric and galvanic methods lead us to the opposite conclusion of vestibular stimulation. They also were unable to demonstrate the decreased labyrinthine sensitivity produced by dimenhydrinate, but they did note that the drug was capable of clinically decreasing the untoward symptoms resulting from morphine usage.

SUMMARY

1. Vestibular responses to morphine, meperidine, codeine, methadon, pantopon and acetylsalicylic acid were studied in man by means of the cold micro-caloric and galvanic stimulation methods.

2. Morphine, meperidine, methadon, and pantopon increased labyrinthine sensitivity while codeine exerted an opposite action. Acetylsalicylic acid produced no changes in vestibular function.

3. Meperidine produced an increased reaction in the cold micro-caloric test in both supine and ambulant patients, but the response in the latter was of greater magnitude.

4. This resultant increased labyrinthine sensitivity may be partially responsible for the higher frequency of dizziness, nausea and vomiting encountered in ambulant patients.

5. Dimenhydrinate is capable of overcoming this resultant increased vestibular response.

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ANNOUNCEMENT OF MEETINGS

The 44th annual meeting of the American Society for Clinical Investigation will be held at the Chalfonte-Haddon Hall, Atlantic City, N. J., on Monday, May 5, 1952, at 9 a.m.

Also at the Chalfonte-Haddon Hall on Tuesday, May 6, and the morning of Wednesday, May 7, 1952, will be the meeting of the Association of American Physicians.

THE EFFECT OF THEOPHYLLINE WITH ETHYLENEDIAMINE (AMINOPHYLLINE) ON CEREBRAL HEMODYNAMICS IN THE PRESENCE OF CARDIAC FAILURE WITH AND WITHOUT CHEYNE-STOKES RESPIRATION [1]

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Cheyne-Stokes respiration, found associated with cardiac failure, has been attributed to cerebral anoxia (1) on the basis of decreased cerebral blood flow, which is depressed in proportion to the decrease in cardiac output (2). Since Aminophylline increases cardiac output (3) and at the same time arrests Cheyne-Stokes respiration (4), it seems logical to conclude that the latter response occurs as a result of improvement of the cerebral circulation. However, Wechsler, Kleiss, and Kety (5) have observed that in normal individuals Aminophylline actually decreases cerebral blood flow. The following study was undertaken in order to clarify this problem. An attempt has been made to evaluate the effect of Aminophylline on the cerebral hemodynamics in 16 patients with cardiac failure, four of whom had Cheyne-Stokes respiration.

METHODS AND MATERIAL

Sixteen cerebral blood flow studies were done on patients with definite clinical and laboratory evidence of congestive heart failure. Their ages ranged from 38 to 70. All showed peripheral edema and signs of pulmonary congestion. The arm to tongue circulation times ranged between 17 and 55 seconds, and the venous pressures taken at the jugular bulb were above 10 cm. water in all but two patients (Table I). Four presented the typical respiratory pattern of Cheyne-Stokes respiration (Table II). Azotemia, with blood urea nitrogens of 34 and 42 mg. per cent, was present in two patients (J. D. and D. B.). This was due to renal disease in the case of J. D. but was apparently secondary to the heart failure in the case of D. B., since there was no evidence of primary kidney disease in the latter. All but four patients had previously received treatment, but had become decompensated because of an inadequate regimen. The hemoglobin was not less than 10 gm. per cent in any of the patients. One of them (M. Y.) presented anxiety manifestations before the studies were started. These symptoms became accentuated after the Aminophylline was given. Additional pertinent clinical data are given in Table I.

The studies were carried out with the patients in the supine position. Two who were too orthopneic to lie supine were tilted to 20 degrees during the entire study. Measurements of pulse rate, respiratory rate, and auscultatory blood pressure were taken during the course of each experiment. Mean blood pressures were calculated on the basis of the diastolic pressure plus one-third of the pulse pressure. A control cerebral blood flow determination was completed employing the nitrous oxide method (6). Nitrous oxide was administered and blood samples collected over a 20 minute period, instead of the usual 10, since equilibrium of nitrous oxide concentration was not always established at 10 minutes due to the decreased blood flow and low rate of arterial delivery of nitrous oxide to the brain. In the patients who exhibited Cheyne-Stokes respiration, when periods of apnea lasted more than 10 seconds, the respiratory rate and rhythm were consciously controlled by the patient at the direction of the investigator who administered the nitrous oxide. This was thought necessary in order to minimize variation in the arterial blood saturation curve of nitrous oxide. The variability seems to have been thus avoided since the arterial blood saturation curves appeared smooth and normal in each instance. Fluctuation in cerebral blood flow within the 10 minute period of study cannot be detected by the nitrous oxide technique since only the average blood flow is determined. Fifteen minutes after the control studies were completed, 0.5 gm. of Aminophylline in 10 ml. of saline was given intravenously over a five minute period. A cerebral blood flow determination was then repeated five minutes after giving the drug. In order to evaluate whether the effect of the drug on the cerebral circulation was transient or active over an extended period of time, a third cerebral blood flow determination was completed in six patients (Table II) 45 minutes after the drug was administered. Cerebral O_2 consumption ($CMRO_2$) and cerebrovascular resistance (CVR) were calculated as previously described (6). The Van Slyke-Neill manometric apparatus (6, 7) was used for blood gas analysis. Aerosol was used in place of saponin (8) as a hemolytic agent.

[1] This study was aided in part by a grant from the National Heart Institute of the National Institutes of Health, United States Public Health Service.

TABLE I

Clinical status and respiratory, pulse rate, and blood pressure responses to Aminophylline

Patient	Sex	Age	Diagnosis	Degree* of failure	Hct.	Circ. time, arm-tongue	R. R.		P. R.		B. P.		M. B. P.			JVP
							C	A	C	A	C	A	C	A	A_1	
B. W.	M	58	SHD, AI	IV	48	35 sec.	28	20	96	94	180/50	160/54	93	89	—	26
E. P.	M	34	Unknown	II	38	34 sec.	27	26	96	96	124/100	110/90	108	97	—	11
E. W.	M	46	AI, RHD, MS	II	37	21 sec.	20	23	77	86	129/37	145/40	68	75	—	18
S. T.	M	65	SHD, AI	II	40	25 sec.	38	40	120	140	180/80	200/110	113	140	—	—
A. J.	F	63	HCVD	I	37	20 sec.	14	14	80	60	148/93	135/82	111	100	113	12
M. Y.	M	44	HCVD	I	48	17 sec.	25	22	92	102	200/130	150/110	153	123	160	18
A. L.	F	59	HCVD	II	36	35 sec.	31	19	74	78	170/115	179/113	133	135	140	16
E. M.	M	46	RHD, AI and MS	IV	55	55 sec.	22	23	104	93	174/0	188/0	58	63	—	17
J. Y.	M	61	SHD, AI	II	—	28 sec.	—	—	—	—	168/59	165/54	95	91	—	14
E. K.	F	56	HCVD	III	42	50 sec.	40	34	136	120	240/140	212/130	173	157	—	17
J. D.	M	56	HCVD	II	36	20 sec.	34	37	110	113	182/142	168/117	155	134	—	9
D. B.	M	56	HCVD	II	36	25 sec.	21	25	94	87	177/107	189/109	130	136	—	10
W. H.	M	38	ASHD	II	39	28 sec.	14	14	96	102	132/85	120/80	101	93	—	12
A. S.	M	57	ASHD	III	34	34 sec.	32	26	100	96	140/68	136/60	92	85	104	20
C. M.	M	70	ASHD	IV	30	38 sec.	36	30	104	106	140/60	136/56	87	83	100	24
C. J.	M	62	HCVD, AI	II	42	35 sec.	42	37	100	96	270/98	235/70	155	125	149	18
Mean		54			40	31 sec.	28	26	99	98	172/85	164/80	114	108	128†	16

* Graded (I-IV) on basis of severity of both objective and subjective manifestations of cardiac failure.

† Mean of control values for some six patients is 122.

SHD—Syphilitic heart disease
HCVD—Hypertensive cardiovascular disease
RHD—Rheumatic heart disease
ASHD—Arteriosclerotic heart disease
AI—Aortic insufficiency
MS—Mitral stenosis

Hct.—Hematocrit
Circ. time—5 ml. Decholin
R. R.—Respiratory rate
P. R.—Pulse rate
B. P.—Blood pressure
M. B. P.—Mean blood pressure (diastolic + pulse pressure/3)
C—Control observations
A—Five minutes after Aminophylline
A_1—Forty-five minutes after Aminophylline
JVP—Control jugular bulb venous pressure, *cm. water*.

RESULTS

The effect of Aminophylline on blood pressure, pulse rate, and respiration in the presence of heart failure is summarized in Table I. The data showing the cerebral hemodynamic response are presented in Table II. Although the blood pressure frequently decreased during the Aminophylline administration, it again became stabilized approximately at the control levels before the cerebral studies were repeated in most of the patients. After stabilization, there was not a statistically significant alteration of the mean blood pressure. It decreased in nine patients (5 mm. or more), increased in four, and in three there was no appreciable change. The effect on the pulse rate was likewise inconsistent. Contrary to the respiratory response in normal individuals (5), the respiratory rate did not increase significantly. However, there was usually an observable increase in the depth of respiration. This difference in response is probably due to the underlying cardiac disease, pulmonary congestion, and the resultant dyspneic state that already existed. The low arterial blood oxygen and carbon dioxide contents that were observed throughout the study were also a reflection of the type of patients studied, *i.e.*, cardiac failure and associated pulmonary congestion (9). In addition, two of them exhibited uremia and four had previously received ammonium chloride as therapy for their disease which undoubtedly served to lower the plasma sodium and therefore the blood CO_2 in these patients. All but four had received mercurial diuretics in the past. Despite the fact that the respiratory rate was not stimulated after the administration of Aminophylline, gaseous exchange within the lungs was somewhat improved. This was reflected in a decrease in the arterial CO_2 content (average decrease = 1.8 vol. per cent) in 14 of the 16 patients. In addition, the maximum concentration of nitrous oxide in the arterial blood

was reached more rapidly after Aminophylline in most of the studies. However, probably due to its lower solubility, the arterial blood oxygen content was not significantly increased. Although the arterial CO_2 decreased after Aminophylline, the jugular venous CO_2 (acute changes in which would more closely reflect changes in tissue CO_2) was not altered.

The control values for cerebral blood flow were below normal (2, 6, 10), *i.e.*, a mean of 45 ml. as compared to a mean of 54 ml. (6, 10) per minute per 100 gm. found in normal individuals. After Aminophylline, the cerebral blood flow became further reduced (mean = 30 ml. per minute per 100 gm.) in all but one patient. This was a result of an increase in cerebrovascular resistance (statistically, $p < 0.01$). In five patients the cerebral blood flow was decreased to 25 ml. per minute or below. This is a reduction of 50 per cent from the normal flow. Even at these low levels there was no obvious alteration of mental acuity.

As the cerebral blood flow decreased after Aminophylline, the oxygen content of the blood flowing from the brain was concurrently reduced (mean of 7.7 vol. per cent reduced to 6.5) resulting in an increased arteriovenous oxygen difference (mean of 7.2 increased to 8.7 vol. per cent). This compensatory phenomenon was incomplete with a resultant significant decrease in cerebral O_2 utilization ($CMRO_2$). The cerebral hemodynamic effects are summarized in Figure 1.

There appeared to be no essential difference in the hemodynamic response immediately or 45 minutes after the administration of Aminophylline (Table II). The patients displaying Cheyne-Stokes respiration (Table II) showed no difference in cerebral hemodynamic response to Aminophylline from that of the other patients in cardiac fail-

TABLE II

Cerebral hemodynamic effect of Aminophylline

Patient	Art. CO_2 (vol. per cent)		Ven. CO_2 (vol. per cent)		Art. O_2 (vol. per cent)		Ven. O_2 (vol. per cent)		A-V O_2 (vol. per cent)			CBF*			$CMRO_2$†		CVR‡	
	C	A	C	A	C	A	C	A	C	A	A_1	C	A	A_1	C	A	C	A
B. W.	35.0	32.4	42.2	44.3	15.2	15.4	6.5	4.1	8.7	11.3	—	31	21	—	2.7	2.4	3.0	4.2
E. P.	48.4	47.7	55.0	56.6	14.7	14.8	7.5	6.5	7.2	8.3	—	57	35	—	4.1	2.9	1.9	2.8
E. W.	34.2	34.9	42.0	42.8	15.6	16.1	7.7	8.0	7.9	8.1	—	37	34	—	2.9	2.8	1.8	2.2
S. T.	36.7	34.3	40.2	42.8	11.8	13.3	7.3	5.8	4.5	7.5	—	61	32	—	2.7	2.4	1.9	4.4
A. J.	41.9	38.5	47.4	47.5	16.6	13.2	9.7	5.2	6.9	8.0	8.8	50	30	24	3.5	2.4	2.2	3.3
M. Y.	31.1	30.8	39.5	39.2	15.6	16.6	6.8	5.8	8.8	10.8	9.7	47	54	48	4.1	5.8	3.3	2.3
A. L.§	29.5	24.6	35.0	33.5	17.6	17.8	10.8	9.7	6.8	8.1	6.2	44	30	36	3.0	2.4	3.0	4.5
E. M.§	20.6	19.8	28.2	29.4	14.7	17.9	6.3	5.3	8.4	12.6	—	39	16	—	3.3	2.0	1.5	3.9
J. Y.§	26.7	26.2	31.0	32.0	12.7	13.2	8.0	8.2	4.7	5.0	—	40	36	—	1.9	1.8	2.4	2.5
E. K.§	24.9	22.8	33.7	31.1	16.6	15.9	9.4	8.4	7.2	7.5	—	58	35	—	4.2	2.6	3.0	4.5
J. D.‖	19.2	18.8	25.8	26.0	11.9	12.8	5.4	4.1	6.5	8.7	—	55	25	—	3.6	2.2	2.8	5.4
D. B.‖	31.7	32.8	38.6	39.4	12.7	12.2	4.3	3.5	8.4	8.7	—	41	33	—	3.4	2.9	3.2	4.1
W. H.¶	33.4	31.0	38.2	39.5	13.4	13.2	7.7	5.0	5.7	8.2	—	51	33	—	2.9	2.7	2.0	2.8
A. S.¶	38.5	33.7	42.5	42.3	17.8	17.6	11.0	9.9	6.8	7.7	6.1	45	30	36	3.1	2.3	2.0	2.8
C. M.¶	41.0	39.0	47.4	47.5	15.7	16.3	7.6	7.2	8.1	9.1	9.7	35	20	22	2.8	1.8	2.5	4.2
C. J.**	30.8	27.5	37.8	37.0	15.9	16.4	7.9	7.4	8.0	9.0	9.7	32	18	20	2.6	1.6	4.8	6.9
Mean	32.7	30.9	39.0	39.4	14.9	15.2	7.7	6.5	7.2	8.7‡‡	8.4	45††	30‡‡	31	3.2	2.6‡‡	2.6	3.8‡‡

C—Control.
A—Five minutes after 0.5 gm. Aminophylline intravenously.
A_1—Forty-five minutes after Aminophylline.
* CBF—Cerebral blood flow, *ml./100 gm./min.*
† $CMRO_2$—O_2 consumption, *ml./100 gm./min.*
‡ CVR—Cerebrovascular resistance, *MBP/CBF/min.*
§ Patient received ammonium chloride as diuretic for prolonged period but discontinued three or more days before present study.
‖ Associated uremia—J. D., 34 mg. %; D. B., 42 mg. %.
¶ Cheyne-Stokes respiration present which was relieved by Aminophylline.
** Cheyne-Stokes respiration not relieved by Aminophylline.
†† Mean value in normal subjects = *54 ml./100 gm./min.* (6, 10).
‡‡ Statistically significant $p < 0.01$.

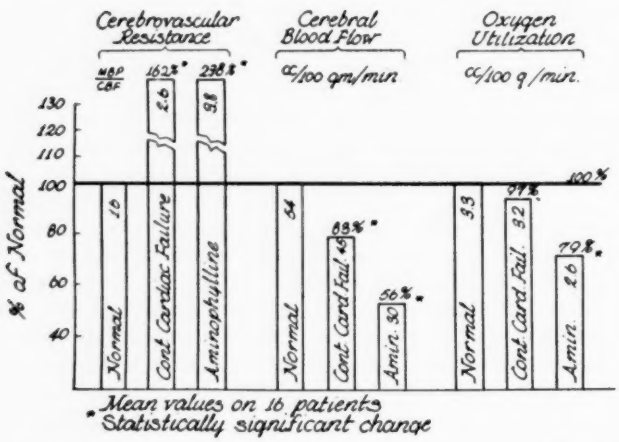

FIG. 1. CEREBRAL HEMODYNAMIC CHANGES IN PATIENTS WITH CARDIAC FAILURE, BEFORE AND AFTER AMINOPHYLLINE, COMPARED WITH THE NORMAL

ure. After the drug, normal rhythmical breathing returned in three of the four patients.

DISCUSSION

The cause of the high initial cerebrovascular resistance and resultant decreased cerebral blood flow in this group of patients exhibiting heart failure is not apparent (2). The highest values for cerebrovascular resistance were seen in those patients with concurrent hypertension. Kety (11) has suggested that increased venous pressure may impede the outflow of blood from the brain. There was no correlation between venous pressure and depressed cerebral flow in the present study. Lowered arterial pCO_2 (not determined in the present study) has also been shown to decrease cerebral blood flow (12). Further studies under these circumstances will be necessary to reach definite conclusions on this point in patients with cardiac failure.

The cerebral blood flow decreased after Aminophylline in all but one patient. The associated acute reduction in arterial CO_2 content, which occurred after the drug was administered in 14 out of 16 patients must reflect, at least qualitatively, a reduction in pCO_2, since only through increased gas exchange in the lungs could this occur so rapidly (five to 10 minutes). Thus, the reduced arterial CO_2 may be a factor in the depression of cerebral blood flow after Aminophylline. If so, it is only a minor factor because when changes in cerebral blood flow of this magnitude due to hyperventilation (12) are compared to the present observations, one may note that decreased CO_2 of a much greater magnitude was required to significantly alter cerebral blood flow. In addition, there is no statistical correlation between reduction in cerebral blood flow and the reduction in arterial CO_2. Moreover, when cerebral blood flow is reduced by acute reduction in arterial CO_2 following hyperventilation, the jugular CO_2 also decreases. In the present group of patients (Table II), the CO_2 content of the blood coming from the brain did not change. Since the jugular blood more closely reflects the state of affairs in brain tissue than does arterial blood, one is led to the conclusion that low CO_2 in the cerebral tissue should be discarded as a responsible factor for the depressed cerebral blood flow after Aminophylline. We therefore conclude, as Wechsler, Kleiss, and Kety (5) have done in similar observations on normal individuals, that the increase in cerebrovascular resistance and decrease in cerebral blood flow is probably a direct effect of Aminophylline on the cerebral vessels. In fact, the small but consistent reduction in arterial CO_2 after Aminophylline, as noted above, could well result from the depressed cerebral blood flow rather than being a causative factor. If so, the increased depth of respiration and improved gaseous exchange would result from the

medullary hemostasis with its accompanying rise in tissue CO_2, thus producing stimulation of the respiratory center (1). Increase in cardiac output with improvement of the pulmonary circulation and the relief of pulmonary edema has not been ruled out as a contributing factor to the decrease in arterial CO_2. The phenomenal increase in cerebrovascular resistance and reduction in cerebral blood flow to levels that have not heretofore been observed indicates that the vasoconstrictive effect of this drug has been superimposed on the increased vascular resistance that is already present in cardiac failure (2). Even in those patients exhibiting hypertension (13), there is a further increase in cerebrovascular resistance after Aminophylline.

In view of the reported cerebral stimulant effect of Aminophylline (1), the decrease in the coefficient of oxygen utilization after the administration of the drug seems paradoxical. Although the arteriovenous oxygen difference increases in an apparent attempt to compensate for the decreased cerebral blood flow, the release of oxygen from the blood is not adequate. This is in contrast to the findings in normal individuals (5), and probably is reflected in the hemoglobin saturation curve of oxygen. Since hemoglobin is inadequately saturated with oxygen in patients with pulmonary congestion, the low pO_2 portion of the curve is more rapidly attained as oxygen is released at the tissue level, especially with cerebral blood flow values in the range of 20 to 30 ml. per minute. Thus, it becomes increasingly difficult for the blood to release an adequate amount of oxygen under these circumstances.

It was anticipated that the mechanism of effect of Aminophylline in arresting Cheyne-Stokes respiration was by improving cerebral blood flow, and thus relieving cerebral anoxia. This was shown not to occur, since those patients with Cheyne-Stokes respiration showed no difference in cerebral hemodynamic response to Aminophylline from the other patients in cardiac failure. The cerebral blood flow and the coefficient of oxygen utilization ($CMRO_2$) both decreased significantly. One would conclude from these observations that the therapeutic effect of Aminophylline in arresting Cheyne-Stokes respiration in the patients studied is probably due to a stimulating effect on the respiratory center. This may be due to a direct effect on the center or an indirect effect by increasing the medullary CO_2 resulting from circulatory stasis.

It is of interest that one patient (M. Y.) showed signs of anxiety which were aggravated by Aminophylline. This is the only patient who showed an increase of oxygen utilization without a decrease in cerebral blood flow. Contrariwise, when the cerebral blood flow decreased to less than half the normal rate in a number of individuals there was no obvious disturbance of cerebration. This fact suggests that consideration of a reduction in cerebral blood flow as a cause of altered cerebral function, rather than merely as an associated finding, must be viewed with caution.

CONCLUSIONS

1. Aminophylline increases the cerebrovascular resistance in patients with cardiac failure. As a result, the cerebral blood flow decreases.

2. Although the arterial blood CO_2 content usually decreases (14 of 16 patients) after Aminophylline, these changes are not great enough to be statistically significant. The jugular CO_2 increases, or it does not change. Therefore, the depression of cerebral blood flow is not a reflection of decreased CO_2 in the brain. It is more likely a direct effect of Aminophylline on the cerebral vessels.

3. Although the arteriovenous oxygen differences increase as a partial compensation for the decreased blood flow, compensation is inadequate. Therefore, the cerebral oxygen consumption appears to decrease when Aminophylline is given to patients with cardiac failure.

4. There is no difference in the over-all cerebral hemodynamic response of patients in cardiac failure manifesting Cheyne-Stokes respiration and those with regular respiration. The arrest of Cheyne-Stokes respiration after Aminophylline cannot be attributed to an increase of the cerebral blood flow. It is probably due to a stimulating effect on the respiratory center. This may be a direct effect or an indirect effect secondary to the depressed cerebral circulation and the resultant rise in medullary tissue CO_2. The fact that this effect of Aminophylline has been observed for at least one hour also indicates that it is not a transient response during the phase of readjustment of the cardiovascular hemodynamics.

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THE EFFECTS OF *l*-EPINEPHRINE AND *l*-NOR-EPINEPHRINE UPON CEREBRAL CIRCULATION AND METABOLISM IN MAN [1]

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Studies comparing the effects of epinephrine and nor-epinephrine, the two sympathomimetic amines which occur naturally in the mammalian body, show that these two closely related substances differ in many of their pharmacodynamic properties. The metabolic rate in man has been shown to increase after epinephrine (1, 2), whereas there is no significant change produced by nor-epinephrine (2). Systemic administration of epinephrine to man has been shown to result in a decrease in total peripheral resistance (1, 3), indicating that vasodilatation has occurred in many portions of the body; in contrast, nor-epinephrine appears to be predominantly vasoconstrictor in its action, for total peripheral resistance is increased (3). These differences may be manifestations of the specific functions which these substances perform in the body; von Euler (4) believes that nor-epinephrine is the sympathetic mediator for general purposes, while epinephrine acts as an adjuvant for more special functions, especially in regard to metabolic actions.

In view of the importance of epinephrine and nor-epinephrine in physiological processes, their occurrence in pheochromocytomas, their use as therapeutic agents, and the possible relation of nor-epinephrine to essential hypertension (3), information regarding their effect on cerebral blood flow and cerebral metabolism is desirable. A preliminary report by Sensenbach, Madison, and Ochs (5) indicates that nor-epinephrine is a powerful cerebral vasoconstrictor. The animal studies upon epinephrine have yielded conflicting results (6–8). Qualitative measurements of cerebral blood flow in man by means of a thermoelectric flow recorder led Gibbs, Gibbs, and Lenox (9) to report an increase in cerebral blood flow following the intravenous injection of adrenalin, probably secondary to the increase in blood pressure.

The present study was undertaken to provide quantitative measurements of cerebral blood flow, vascular resistance and oxygen consumption in man during the intravenous infusion of synthetic *l*-epinephrine or *l*-nor-epinephrine.

METHODS

Subjects varied in age from 19 to 50 years. They were either volunteers or hospital patients with apparently normal cardiovascular systems. Two subjects (T. I. and T. C.) who received epinephrine served also as subjects for the nor-epinephrine study at a later date. Cerebral blood flow (CBF) was determined by the nitrous oxide method (10). Blood pressure and pulse rate were obtained by means of a Lilly capacitance electromanometer (11, 12) attached to the needle in the femoral artery, and recorded continuously by means of a Brush direct-inking oscillograph except when arterial blood samples were being drawn. Mean arterial blood pressure (MABP) was obtained by either a damped mercury manometer or by electrical integration of the pressure pulse curves from the Lilly manometer. Cerebral metabolic rate in terms of cerebral oxygen consumption (CMR_{O_2}) and cerebrovascular resistance (CVR) were calculated as previously described (10). Blood gas analyses were made by the manometric technique of Van Slyke and Neill (13). Measurements of blood pH were made anaerobically at room temperature by means of a glass electrode and a Cambridge potentiometer and corrected to 37° C. by the factors of Rosenthal (14). Values for blood carbon dioxide tension were calculated by means of the nomograms of Peters and Van Slyke (13). Total hemoglobin concentration of arterial blood was determined by a modification of the method of Evelyn and Malloy (15).

Before the control measurements, an intravenous infusion of physiological saline was begun. Subsequently the infusion was changed to one containing either synthetic

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l-epinephrine or *l*-nor-epinephrine,[2] 4 or 8 micrograms of drug per cc. of saline. The rate of drug infusion was adjusted until the desired pressor response was acquired and maintained or until undesired side effects occurred. When a steady state had been attained, a second set of blood flow determinations was made. The total quantity of drug and the rate at which it was administered are indicated for each subject in Table I.

RESULTS

The data are presented in Tables I, II, and III. Values for systolic and diastolic blood pressure and pulse rate were obtained for each blood flow determination by averaging measurements secured at five intervals on the continuous record. A maximum and minimum of blood pressure were selected at each of these intervals to take into account the effects of the respiratory cycle on blood pressure.

Effects of nor-epinephrine

Subjective sensations, except for a feeling that the heart was beating more forcefully, were notably absent during the infusions of nor-epinephrine. The skin and mucous membranes became pale. The pressor effect was more marked than with epinephrine and was produced with a much lower dose. Slowing of the pulse rate accompanied the rise in blood pressure in every case.

The hemoglobin concentration of arterial blood was increased significantly; this might be a result of fluid dislocation from the vascular bed or of emptying of red cell reservoirs. Arterial carbon dioxide tension was decreased, probably as a result of hyperventilation although this was not evident by inspection. These alterations in carbon

[2] *l*-Epinephrine as "Suprarenin" and *l*-nor-epinephrine as "Levophen" or "Levophed" were provided through the courtesy of Winthrop-Stearns.

TABLE I

Effects of l-nor-epinephrine and l-epinephrine on blood pressure and pulse rate

Subject	Age	Sex	Dose Total	Dose Rate	Syst. & Diast. Blood Pressure C+	Syst. & Diast. Blood Pressure E‡	Pulse Rate C	Pulse Rate E
			μg.	μg./min.				
l-nor-epinephrine								
(1) H. A.	39	M	770	22	128/82	160/81	90	59
(2) E. H.	50	F	312	10	141/77	185/84	112	79
(3) A. H.	48	F	464	16	138/80	185/85	88	66
(4) L. W.	38	F	576	16	142/81	184/86	80	53
(5) M. S.	36	M	760	25	126/73	165/77	65	52
(6) T. C.	24	M	500	17	106/68	134/81	84	63
(7) T. I.	24	M	840	28	110/72	150/89	65	51
(8) L. H. I.	19	M	200	6	116/61	173/89	65	52
(9) N. Y.	22	M	300	9	125/78	172/97	74	61
Mean	33.3		524.7	16.6	126/75	168/85	80.3	59.6
Stand. error	±3.8						±5.2	±3.0
p*							<0.001	
l-epinephrine								
(10) A. M.	34	F	1732	73	135/81	145/79	100	116
(11) T. I.	24	M	800	47	113/73	159/91	81	91
(12) T. C.	24	M	664	33	112/65	175/84	75	72
(13) J. F.	25	M	976	34	109/79	134/76	71	83
(14) L. H.	25	M	800	30	103/65	126/74	54	78
(15) A. Mc.	22	M	480	19	117/67	132/63	70	72
(16) D. F.	19	M	480	22	131/72	173/75	97	115
Mean	24.7		847.4	36.9	117/72	149/77	78.3	89.6
Stand. error	±1.7						±6.1	±7.1
p*							<0.02	

* Determined from the t value obtained by dividing the mean of the individual changes by its standard error.

\+ Control period - Saline infusion.

‡ Experimental period - Drug infusion.

TABLE II

Effect of l-nor-epinephrine and l-epinephrine on blood constituents

	Subject	Hemoglobin Concentration Gms. %		O_2 Content vol. %				CO_2 Content vol. %				Blood pH				CO_2 Tension mm. Hg			
				Arterial		Int. Jugular		Arterial		Int. Jugular		Arterial		Int. Jugular		Arterial		Int. Jugular	
		C+	E‡	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
l-Nor-Epinephrine	(1) H. A.	15.17	15.51	19.75	19.25	12.08	10.79	49.79	44.22	56.75	52.66	7.41	7.42	7.37	7.37	42	38	52	48
	(2) E. H.	10.48	10.39	13.89	13.80	8.47	8.91	53.08	51.04	56.97	56.02	7.43	7.42	7.38	7.37	40	40	48	48
	(3) A. H.	10.07	10.07	13.47	13.72	7.00	7.37	51.17	48.60	56.91	55.17	7.42	7.43	7.36	7.36	40	37	49	48
	(4) L. W.	10.72	10.56	14.37	14.61	7.89	7.48	56.23	51.92	61.75	59.05	7.41	7.43	7.37	7.37	44	40	53	50
	(5) M. S.	14.23	15.07	20.20	19.18	13.08	12.99	49.19	53.28	56.83	59.73	7.38	7.38	7.32	7.36	44	48	57	56
	(6) T. C.	13.56	13.94	16.23	16.66	12.53	12.39	48.87	45.34	52.68	49.83	7.38	7.38	7.37	7.36	43	40	48	46
	(7) T. I.	13.64	14.64	17.27	18.88	11.91	11.62	48.2[illegible]	42.75	52.95	49.64	7.36	7.34	7.34	7.32	44	42	50	50
	(8) L. H. I.	16.16	16.73	19.88	20.96	13.37	13.44	48.37	45.35	54.29	52.07	7.35	7.38	7.30	7.30	46	42	57	55
	(9) N. Y.	17.16	17.64	21.86	22.51	15.75	16.53	47.53	42.93	53.31	49.19	7.39	7.41	7.35	7.37	43	38	52	46
	Mean	13.47	13.84	17.44	17.73	11.34	11.28	50.27	47.27	55.83	53.71	7.39	7.40	7.35	7.35	42.9	40.5	51.8	49.7
	Stand. error	±0.85	±0.95	±1.04	±1.06	±0.87	±1.00	±0.93	±1.34	±0.96	±1.33	±.01	±0.01	±0.01	±0.01	±0.7	±1.1	±1.2	±1.2
	p*	< 0.05		> 0.6		> 0.7		< 0.02		< 0.02		> 0.2		> 0.7		< 0.05		< 0.02	
l-Epinephrine	(10) A. M.	12.72	12.35	16.91	16.61	10.07	9.82	47.85	39.01	57.42	45.76	7.35	7.34	7.33	7.32	44	36	54	44
	(11) T. I.	13.10	13.86	18.92	20.51	13.32	14.25	48.12	44.75	53.27	50.88	7.44	7.45	7.33	7.34	38	34	52	49
	(12) T. C.	12.18	13.01	17.12	18.08	11.15	13.48	46.55	44.86	51.58	49.92	7.45	7.41	7.39	7.37	35	37	44	45
	(13) J. F.	13.48	13.86	19.38	20.12	11.93	11.90	51.23	44.55	58.02	52.40	7.39	7.47	7.37	7.33	44	33	53	51
	(14) L. H.	15.15	15.37	20.53	21.74	11.24	11.93	45.09	40.67	53.15	49.72	7.37	7.36	7.30	7.31	41	38	55	50
	(15) A. Mc.	16.90	16.90	21.16	22.45	15.80	16.37	43.42	41.57	48.92	47.27	7.33	7.33	7.28	7.24	43	42	57	57
	(16) D. F.	15.08	15.38	19.56	20.23	11.67	12.07	43.90	41.61	51.19	49.70	7.34	7.32	7.28	7.30	42	42	56	52
	Mean	14.09	14.39	19.08	19.97	12.17	12.83	46.59	42.43	53.36	49.38	7.38	7.38	7.33	7.32	41.0	37.4	53.0	49.7
	Stand. error	±0.63	±0.59	±0.60	±0.77	±0.71	±0.79	±1.04	±0.87	±1.25	±0.84	±0.02	±0.02	±0.02	±0.02	±1.3	±1.3	±1.6	±1.7
	p*	> 0.1		< 0.01		> 0.05		< 0.01		< 0.05		> 0.9		> 0.3		> 0.05		> 0.05	

* Determined from the t value obtained by dividing the mean of the individual changes by its standard error.

\+ Control period - Saline infusion.

‡ Experimental - Drug infusion.

TABLE III

Effects of l-nor-epinephrine and l-epinephrine on cerebral circulation and oxygen consumption

	Subject	MABP mm. Hg.		CBF cc/100gm/min.		CVR mm. Hg / cc/100gm/min.		$(A\text{-}V)O_2$ vol. %		$CMR\ O_2$ cc/100gm/min.		RQ (cerebral)	
		C+	E‡	C	E	C	E	C	E	C	E	C	E
l-Nor-Epinephrine	(1) H. A.	92	115	45	40	2.0	2.9	7.67	8.46	3.5	3.4	0.91	1.00
	(2) E. H.	100	123	61	49	1.6	2.5	5.42	4.89	3.3	2.4	0.72	1.02
	(3) A. H.	86	112	56	52	1.5	2.2	6.47	6.35	3.6	3.3	0.89	1.03
	(4) L. W.	100	130	60	56	1.7	2.3	6.48	7.13	3.9	4.0	0.85	1.01
	(5) M. S.	95	118	70	72	1.4	1.6	7.15	6.19	5.0	4.5	1.07	1.04
	(6) T. C.	84	102	80	80	1.1	1.3	3.70	4.27	3.0	3.4	1.03	1.05
	(7) T. I.	86	106	71	59	1.2	1.8	5.36	7.28	3.8	4.3	0.88	0.95
	(8) L. H. I.	83	124	69	52	1.2	2.4	6.51	7.52	4.5	3.9	0.91	0.90
	(9) N. Y.	93	123	41	40	2.3	3.1	6.11	6.01	2.5	2.4	0.95	1.04
	Mean	91.0	117.0	61.4	55.6	1.56	2.23	6.10	6.46	3.68	3.51	0.91	1.00
	Stand. error	±2.2	±3.0	±4.2	±4.5	±0.13	±0.20	±0.39	±0.44	±0.25	±0.25	±0.03	±0.02
	p*	<0.001		<0.05		<0.001		>0.2		>0.3		<0.05	
l-Epinephrine	(10) A. M.	103	109	47	49	2.2	2.2	6.84	6.79	3.2	3.3	1.00	0.99
	(11) T. I.	90	133	60	85	1.5	1.6	5.60	6.26	3.4	5.3	0.90	0.98
	(12) T. C.	88	128	49	68	1.8	1.9	5.97	4.60	2.9	3.1	0.84	1.10
	(13) J. F.	107	120	56	65	1.9	1.8	7.45	8.22	4.2	5.3	0.91	0.96
	(14) L. H.	78	90	35	44	2.2	2.0	9.29	9.81	3.2	4.0	0.97	0.92
	(15) A. Mc.	85	86	50	59	1.7	1.5	5.36	6.08	2.7	3.6	1.03	0.94
	(16) D. F.	85	95	54	55	1.6	1.7	7.89	8.16	4.3	4.5	0.92	0.98
	Mean	90.9	108.7	50.1	60.7	1.84	1.81	6.91	7.13	3.41	4.16	0.94	0.98
	Stand. error	±3.9	±7.1	±3.0	±5.2	±0.10	±0.09	±0.53	±0.65	±0.14	±0.34	±0.02	±0.02
	p*	<0.05		<0.02		>0.6		>0.4		<0.05		>0.3	

* Determined from the t value obtained by dividing the mean of the individual changes by its standard error.
\+ Control period - Saline infusion.
‡ Experimental period - Drug infusion.

dioxide tension were not of sufficient magnitude to have a significant effect on cerebrovascular dynamics (16).

Despite the marked rise in mean arterial blood pressure from 91 to 117 mm. Hg, cerebral blood flow decreased from 61 to 56 cc./100 gm./min. ($p < 0.05$), apparently because of an increase in cerebrovascular resistance from 1.6 to 2.2 resistance units ($p < 0.001$). An increase in resistance occurred in each of the individuals studied. There was no significant alteration in cerebral metabolism.

Effects of epinephrine

The administration of epinephrine was often accompanied by palpitation, tremor of the hands, or a sense of excitement or apprehension. Cutaneous and mucosal pallor was usually marked. The pulse rate, although significantly increased during the blood flow determinations, accelerated more at the beginning and immediately after the termination of the infusions when the blood pressure elevation was less prominent.

Mean arterial blood pressure increased from 91 to 109 mm. Hg, and cerebral blood flow rose from a mean of 50 to 61 cc./100 gm./min. ($p < 0.02$). This increase in cerebral blood flow is accounted for by the lack of significant change in cerebrovascular resistance during the period of hypertension. Of particular interest was the augmentation of cerebral oxygen consumption from 3.4 to 4.2 cc. O_2/100 gm./min. ($p < 0.05$).

DISCUSSION

These studies show that *l*-epinephrine and *l*-nor-epinephrine act differently with respect to both cerebral circulation and cerebral metabolism. The significant increase in cerebral oxygen consumption accompanying epinephrine infusions is of particular interest. Such observations indicate that the brain shares in the metabolic augmentation which is produced in the body as a whole by epinephrine. One subject (No. 11), in whom marked sensations of apprehension followed administration of the drug, had an increase in cerebral oxygen consumption from 3.4 to 5.3 cc. O_2/100 gm./min. The fact that a state of anxiety or apprehension may be associated with a greatly augmented cerebral oxygen consumption has been

pointed out by Kety (17), who reported a value of 5.0 cc. O_2/100 gm./min. under such circumstances when previous resting values were 3.4, 3.9, 3.2, and 4.2 cc. O_2/100 gm./min. These observations lead to speculation as to whether the increased cerebral oxygen consumption during apprehension is due to the liberation of endogenous epinephrine. Nor-epinephrine, which does not appear to produce such psychic effects, is not associated with such increases in cerebral oxygen consumption. These findings controvert the suggestion made by Scheinberg (18) that cerebral metabolism normally functions at nearly its maximal rate and that "no effective means of increasing cerebral metabolism in man have been found."

It should be pointed out that a rise in mean arterial blood pressure does not necessarily result in an increase in cerebral blood flow. Despite a marked rise in blood pressure caused by nor-epinephrine, there was a decrease in blood flow because of the simultaneous increase in cerebrovascular resistance. Since an important aspect of cerebral homeostasis consists in an adjustment of circulation to local metabolic needs (17), it is providential that epinephrine, which increases cerebral metabolism, does not simultaneously increase cerebrovascular resistance. It is possible that epinephrine might exert a vasoconstricting action on the cerebral vessels which is balanced by a vasodilator effect resulting from an accumulation of substances produced by the increased cerebral metabolism. However, the higher oxygen content and lower carbon dioxide tension existing in the internal jugular blood during the drug infusion indicate that cerebral blood flow is more than adequate, which prevents metabolic products from accumulating. The unchanged cerebrovascular resistance following the administration of epinephrine seems to indicate that if there were vasoconstriction from this drug, it was not sufficient to do more than overcome any passive dilatation resulting from increased systemic blood pressure.

Experiments upon anesthetized animals have indicated that epinephrine can cause cerebral vasoconstriction (6, 7). Our results may differ because of species difference, the state of consciousness, composition of the drug, or mode of administration. Both of the drugs used in this study were synthetic preparations, believed to be free from contamination by each other. On the other hand, chemical analysis of U.S.P. grade epinephrine has revealed that the nor-epinephrine content of epinephrine from animal sources may be as high as 18.5% (19); such contaminated epinephrine may have been used in earlier studies. Although the quantities of nor-epinephrine contained in natural epinephrine do not appear to alter significantly the hemodynamic actions of the latter drug (20), one cannot be assured such is the case in relation to cerebrovascular actions.

In this study the drug was given by continuous intravenous infusion. The concentration reaching the cerebral vessels under such circumstances is undoubtedly much lower than was attained in those animal experiments in which the drug was applied topically (7) or injected into the carotid artery (6) and in which cerebral vasconstriction was reported.

The question may be raised whether epinephrine could cause cerebral vasoconstriction if given in sufficient quantity to produce a pressor effect equivalent to that elicited by nor-epinephrine. Side effects such as arrhythmias, palpitation, or apprehension placed limitations on the amount of epinephrine which could be used in this study. However, in two individuals (Nos. 11 and 12) to whom sufficient epinephrine was given to produce pressor effects equal to or greater than those obtained in any of the subjects receiving nor-epinephrine, there was no significant rise in cerebrovascular resistance. We conclude that epinephrine, given systemically and within the limits of physiological tolerance, does not produce cerebral vasoconstriction in man.

Our data for nor-epinephrine are essentially in agreement with those of Sensenbach, Madison, and Ochs (5). The primary difference is a lack of a statistically significant decrease in cerebral blood flow in their series. This might be attributed to the variability of absorption of the drug from an intramuscular depot. In our series, a continuous intravenous infusion associated with continuous blood pressure recording allowed us to achieve a constant pressor effect.

Goldenberg and his co-workers (3) have suggested that a disturbed balance between nor-epinephrine and epinephrine might be an etiologic factor in essential hypertension. Evidence supporting this view is the fact that essential hypertension and infusions of nor-epinephrine are both

associated with increases in total peripheral resistance and blood pressure without any increase in cardiac output. Evidence against this view consists of the finding that nor-epinephrine decreases forearm blood flow and produces facial pallor and bradycardia, manifestations which are not characteristic of essential hypertension (*21*). Our data show another similarity between essential hypertension (*22*) and the state produced by infusions of nor-epinephrine, namely that both are associated with an increase in cerebrovascular resistance. Although cerebral blood flow was significantly decreased by nor-epinephrine, it still remained within normal limits, as is the case with essential hypertension (*22*).

Nor-epinephrine has been proposed as a useful and potent pressor drug (*23*), and is now available for clinical use. If the vascular beds of other vital organs such as the heart, liver, and kidneys respond with vasoconstriction as do the vessels of the brain, situations could occur where therapy with this and similarly acting drugs might be unsatisfactory or actually harmful. For example, if sympathetically induced vasoconstriction through most of the body was already maximal during an episode of hypotension, nor-epinephrine, which is unquestionably a cerebral vasoconstrictor, might increase cerebrovascular resistance out of proportion to the increase in perfusion pressure so that cerebral blood flow might actually decrease. For this reason, nor-epinephrine and related drugs should be evaluated not only by measurement of their pressor effects but also by measurement of blood flow through vital organs in normotensive and hypotensive states.

SUMMARY

1. The effects of continuous intravenous infusions of synthetic *l*-epinephrine and *l*-nor-epinephrine upon arterial blood pressure and pulse rate, arterial and internal jugular blood gases, and cerebral blood flow, oxygen consumption, and vascular resistance have been studied in man.

2. Epinephrine produced an increase in mean arterial blood pressure and cerebral blood flow without a significant change in cerebrovascular resistance. In addition there was a significant increase in cerebral oxygen consumption.

3. Nor-epinephrine, a more potent pressor drug, produced a marked increase in cerebrovascular resistance, and a decrease in cerebral blood flow despite a substantial increase in mean arterial blood pressure. Cerebral oxygen consumption was not significantly altered. The significance of these findings in relation to the clinical use of nor-epinephrine is discussed.

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THE VOLUME OF DISTRIBUTION OF SODIUM THIOSULFATE AS A MEASURE OF THE EXTRACELLULAR FLUID SPACE [1]

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The importance of total body fluid and electrolytes and their distribution in the body cell mass has been increasingly emphasized in the care of acutely and chronically ill patients. In order to determine distribution of water and electrolytes, a convenient and accurate technique for the estimation of the extracellular fluid space is essential.

Moore (1) has demonstrated that the chemical dissection of the patient (to determine total body composition) is feasible by the dilution of radioactive and stable isotope tracers used in conjunction with a means for measuring the extracellular fluid. While techniques such as deuterium dilution, tritium dilution, and antipyrine dilution for the *in vivo* measurement of total body water can be checked for absolute accuracy against desiccation and specific gravity measurements (2), no such criterion for the accuracy of extracellular fluid measurements is available. Evaluation of extracellular fluid techniques must depend upon their reproducibility and compatibility with microanatomical studies and estimations of the volume of distribution of normal extracellular constituents.

In addition to interstitial and plasma water, the extracellular compartment must be considered to include the cerebrospinal fluid, water in the gastrointestinal tract, in glandular lumina, and bone matrix water. No technique is at present available which has been proven to measure the absolute volume of the entire compartment. Indeed at certain points the exact boundaries of the "compartment" defy anatomical definition. In the absence of an absolute standard for extracellular fluid measurement, reasonable criteria for assessing a particular method would be that it must (*a*) include a significant fraction of the compartment, (*b*) vary in proportion to changes in the extracellular space, (*c*) be insensitive to changes in cell permeability, and (*d*) be reproducible and technically convenient.

Previous studies by Newman, Gilman, and others (3–5) employing sodium thiosulfate have demonstrated (*a*) that its equilibrium volume of distribution is in the range of the extracellular fluid volume, (*b*) that its rate of disappearance after equilibrium of distribution is proportional to its concentration, (*c*) that it diffuses rapidly, and (*d*) that it is metabolized slowly and at an exponential rate (4).

These properties suggested that the thiosulfate ion would prove to be a suitable and convenient measure of the extracellular fluid by a technique not requiring either urine collections or a long period of constant infusion such as is necessary with inulin.

The present study was undertaken to examine the volume of distribution of sodium thiosulfate observed after a brief single injection and the collection of several serial blood samples over a short interval of time.

METHODS

Seven adult mongrel dogs ranging from 8.5–14.4 kgm. in weight were used in the preliminary experiments. Normal healthy male adult humans, ages 18–30, were studied for the arterio-venous difference, reproducibility and erythrocyte penetration studies, and hospital patients as specifically described in the next section were studied in febrile and edema states.

Twenty-five to 30 ml. of sterile 10% commercial hydrated sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)[4] were injected into the dogs in one to two minutes. For human studies, 12 gm. of sterile sodium thiosulfate were diluted

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in 120 ml. of sterile pyrogen free water, 20 ml. withdrawn for determination of density and titration, and the remaining material administered by infusion after careful weighing to 0.01 gm. On completion of the 10–12 minute infusion, the infusion set was washed through three times with saline. Studies on a fourth rinse reveal less than 0.01% of the dose remaining.

Four to 12 blood samples were withdrawn from an indwelling needle (Cournand) in the femoral artery and venous blood from the femoral vein in dogs and the antecubital veins in man.

Samples in the preliminary studies were analyzed according to the indirect iodometric macro-method of Newman (3). In later studies it was found that a microtechnique afforded equal accuracy and required much less blood, thus permitting more samples and better definition of the curve without excessive blood loss. This technique [5] was modified from that suggested by Newman.

Calculation of the thiosulfate space

Venous serum concentrations are plotted semilogarithmically against the mid-time of sample collection and the equilibrium concentration obtained by extrapolation to the time of commencement of infusion (zero time). The plotted values must demonstrate linearity within the limits of error of the experiment or extrapolation is invalid. The extrapolated concentration is divided into the dose injected to obtain the volume of distribution.[5]

RESULTS

1. *Accuracy of chemical technique*

Duplicate samples were reproducible within an average range of 4 parts per 1,000. Recovery from plasma by this method averaged 96.5% with a range of 93.3–100%. Recovery from water averaged 98% ranging from 95–100%.

[5] See Appendix II.

2. *Expected accuracy in absolute quantities*

Accuracy in absolute quantities was estimated by a "dummy" measurement of 7,052 ml. This was done to indicate whether significant changes in volume of distribution might be masked by the error of the technique. Calculated values in quintuplicate averaged 7,265.3 ± 58.7 ml. This is an error of 213 ml. or 3.0%.

3. *Erythrocyte penetration*

Studies on erythrocytes incubated *in vitro* for 90 minutes at room temperature with oxygenation and gentle agitation indicated complete penetration of red cell water by sodium thiosulfate.

Studies *in vivo* were made by drawing blood 70 minutes after injection of thiosulfate, determining the hematocrit corrected for 3% trapped plasma, and splitting the sample, one-half of which was used for calculating plasma concentration. The other half was used for determining whole blood concentration, after hemolysis induced by alternate freezing and thawing. Table I summarizes the data obtained. Since at equilibrium of distribution, with complete penetration, the concentration of thiosulfate in both plasma water and red cell water will equal the concentration in whole blood water, the ratio of thiosulfate concentration in erythrocyte water to thiosulfate concentration in whole blood water was used as the index of penetration. In these experiments the ratios so obtained were 0.92, 0.42, and 0.62.

4. *Linearity of total clearance*

Preliminary studies were made on dogs employing an injection time of one to two minutes with

TABLE I

Penetration of thiosulfate into erythrocytes in vivo

Subject	Corr. hematocrit	Plasma $S_2O_3^=$	Whole blood $S_2O_3^=$	Plasma* water $S_2O_3^=$	Whole† blood water $S_2O_3^=$	Erythrocyte‡ water $S_2O_3^=$	EWT/WBWT§
		mgm./ml.	*mgm./ml.*	*mgm./ml.*	*mgm./ml.*	*mgm./ml.*	
D. H.	36	0.133	0.116	0.145	0.140	0.128	0.92
D. H.	39	0.142	0.100	0.154	0.121	0.051	0.42
J. R.	47.5	0.083	0.058	0.090	0.073	0.045	0.62

* Plasma water thiosulfate concentration = concentration of thiosulfate in plasma ÷ 0.92.

† Whole blood water thiosulfate concentration = concentration of thiosulfate in whole blood ÷ (0.92 [plasma volume] + 0.67 [red cell volume]).

‡ Erythrocyte water thiosulfate concentration = (thiosulfate content of 1 ml. whole blood − thiosulfate content in plasma fraction of 1 ml. whole blood) ÷ 0.67 × red cell volume per ml. of whole blood.

§ EWT/WBWT = ratio of the concentration of thiosulfate in erythrocyte water to the concentration in whole blood water.

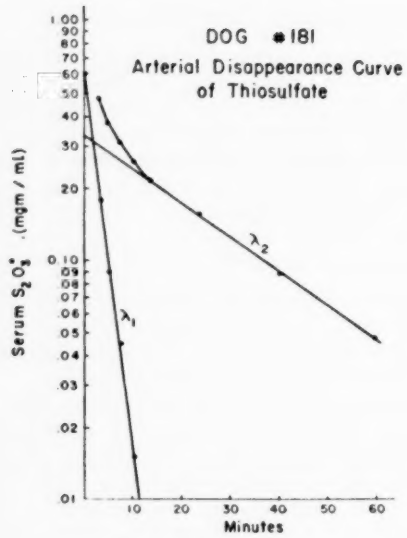

FIG. 1

Subject was a 13.0 kgm. dog. Twenty-five ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were injected into the femoral vein in one minute. Samples were withdrawn from the femoral artery through an indwelling (Cournand) needle and serum thiosulfate concentrations plotted against time. Curve analysis was by standard subtraction technique (11).

12 samples obtained from one to 70 minutes after the beginning of injection. In human subjects thiosulfate was administered by infusion lasting eight to 12 minutes and four to 12 arterial and/or venous samples were obtained. In 15 curves on seven dogs and 18 curves on 14 humans, linearity was demonstrated within the limits of error of the experiment. Typical examples of the resulting curves are presented in Figures 1 and 2.

TABLE II

Comparison of thiosulfate space determined simultaneously from arterial and venous blood levels

Subject	Arterial		Venous		Difference	
	Liters	% body weight	Liters	% body weight	Liters	% body weight
R. H. C.	10.88	17.5	10.00	16.1	−0.88	−1.4
R. H. C.	11.29	18.5	10.86	17.8	−0.45	−0.7
R. H.	10.50	13.3	10.33	13.0	−0.17	−0.3
J. R.	11.88	17.8	11.88	17.8	0.00	−0.0
T. D. B.	13.83	18.1	13.17	17.2	−0.66	−0.9
Mean	11.68	18.0	11.05	17.2	0.63	−0.8

5. Effect of infusion time

Infusion times in six reproducibility experiments are shown in Table IV. In general, infusions were set to run 10–12 minutes. Short infusions were avoided to minimize the increased renal loss with very high plasma concentration prior to equilibrium and because of the possibility of nausea and vomiting. The largest variation in infusion time was between 7.4 minutes on the first measurement and 12.6 minutes on the second measurement of one individual.

6. Arterio-venous difference

Samples in all of the preliminary studies on dogs were drawn through an indwelling needle (Cournand) in the femoral artery. In transferring attention to man it was of obvious advantage to use venous rather than arterial blood. Two studies on dogs showed a marked lag in the fall of venous as compared with arterial concentration. Typical simultaneous arterio-venous curves in man are shown in Figure 3, and volumes of distribution

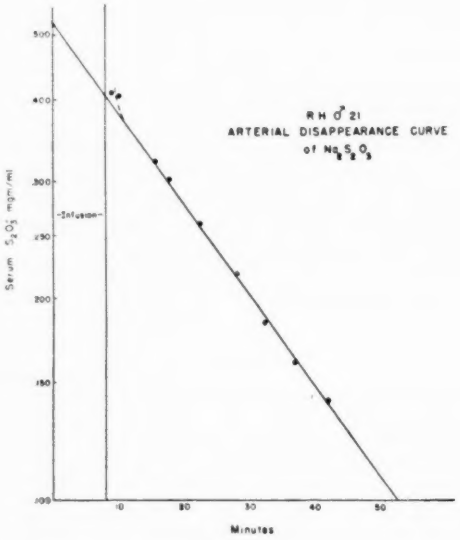

FIG. 2

Subject was a healthy male student, age 21. 102 ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were administered by infusion over 7.4 minutes. Samples were drawn from the femoral artery by an indwelling (Cournand) needle and serum thiosulfate concentrations plotted logarithmically against time.

TABLE III

Volume of distribution of sodium thiosulfate in dogs

Dog no.	Body weight	Thiosulfate space	
	kgm.	*liters*	*% body weight*
195	8.5	2.0	23.6
203	14.4	4.1	28.1
204	13.7	3.6	27.2
223	12.8	3.0	23.2
224	12.4	3.1	24.6
181	11.5	2.3	20.3
182	12.7	3.1	24.4
Mean	12.4	3.0	24.4

determined simultaneously from arterial and venous samples in four healthy young adult male humans are tabulated in Table II. The average volume of distribution was 0.63 liter (0.8% of body weight) smaller by venous concentration. The largest discrepancy in volume distribution was 0.9 liter or 1.4% of the body weight. At any one time the venous concentration of thiosulfate is higher than the arterial after distribution equilibrium has taken place (Figure 3). In addition, although not marked in every case, the venous concentration fell more slowly than the arterial. This discrepancy in rate of fall of thiosulfate concentration is less evident in the experiment given in Figure 3 than in some of the others studied.

7. *Volume of distribution and reproducibility*

The volumes of distribution of thiosulfate as determined in seven dogs under similar conditions are presented in Table III. The average value was 3 liters or 24.4% of body weight. Table IV shows the volumes of distribution in six healthy male adult humans studied on two occasions at various time intervals. As far as possible, subjects

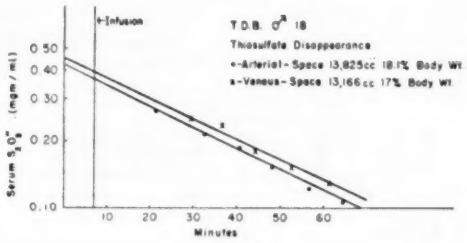

FIG. 3

Subject was a healthy male student, age 19. 101 ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were administered by infusion over 6.9 minutes. Arterial samples were drawn from the femoral artery using an indwelling (Cournand) needle. Venous samples were drawn from the antecubital veins and all serum thiosulfate concentrations plotted logarithmically against time.

in a "steady state" were chosen and no illness occurred between measurements. The average thiosulfate space was 12.2 liters or 16.6% of body weight and the average interval change of 0.42 liter or 0.38% of body weight is smaller than the expected general error of the measurement.

8. *Volumes of distribution in febrile patients*

Sensitivity of the thiosulfate ion to changes in cell permeability was tested in three hospital patients with febrile illnesses. Volumes of distribution are presented in Table V. An effort was made to choose patients free from any chronic underlying disease or complication which might affect fluid or electrolyte distribution or kinetics. All were adequately hydrated at the time of measurement but were not edematous. Volumes of distribution in these patients were 17.9, 18.4, and 15.9% of body weight.

TABLE IV

Volume of distribution and reproducibility of thiosulfate space in adult male humans

Subject	Age	First measurement							Second measurement							Difference		
		Date	Infusion time	Body weight	Surface area	Thiosulfate space			Date	Infusion time	Body weight	Surface area	Thiosulfate space					
	yrs.		*min.*	*kgm.*	M^2	*liters*	% *b. wt.*	L/M^2		*min.*	*kgm.*	M^2	*liters*	% *b. wt.*	L/M^2	*liters*	% *b. wt.*	L/M^2
R. H. C.	30	12/8/50	13.6	62.2	1.68	10.00	16.1	5.95	1/11/51	16.5	61.1	1.67	10.68	17.8	6.50	+0.68	+1.7	+0.55
J. R.	18	1/16/51	11.0	66.8	1.86	11.88	17.8	6.39	2/9/51	10.8	67.6	1.87	11.78	17.4	6.30	−0.10	−0.4	−0.09
T. D. B.	19	1/19/51	6.9	76.2	1.99	13.17	17.2	6.62	2/12/51	11.3	75.0	1.97	11.93	15.9	6.06	−1.24	−1.3	−0.56
G. C.	18	1/23/51	8.4	82.6	2.06	13.74	16.6	6.67	2/5/51	11.6	82.9	2.07	13.74	16.5	6.64	0.00	−0.1	−0.03
F. R. C.	21	1/22/51	12.4	76.7	2.00	12.48	16.3	6.24	2/8/51	11.1	75.2	1.98	11.79	15.7	5.95	−0.69	−0.6	−0.29
R. H.	21	1/26/51	12.6	81.2	2.04	13.39	16.5	6.56	3/6/51	7.4	80.6	2.03	12.05	14.9	5.94	−1.3	−1.6	−0.65
Mean			10.8			12.44	16.75	6.41		11.4			12.02	16.37	6.23	−0.42	−0.38	−0.18

TABLE V

Volume of thiosulfate distribution in febrile patients

Patient	Sex	Age	Diagnosis	Temp. range	Body weight	S_2O_3 space	
		yrs.		*° F.*	*kgm.*	*liters*	*% body wt.*
M. R.	F	18	Infectious mononucleosis	103	54.8	10.0	17.9
E. C.	M	68	Bronchopneumonia	101.8–103.5	63.2	11.5	18.4
S. K.	M	27	Post-lithotomy fever	101.4–102.4	72.0	11.5	15.9

9. Edema

As a first step in evaluating the relative accuracy of thiosulfate dilution as a measure of the extracellular fluid volume, patients with obvious edema were studied. Table VI lists the determinations carried out in five such patients. In these subjects the thiosulfate volume of dilution ranged from 14.6 to 20.7 liters, corresponding to 21.5–28.7% of the body weight. All of these values are considerably greater than those found in any of the normals. In two of the five subjects the volumes obtained were close to twice that obtained in the normal. In these instances the thiosulfate space has changed in the same direction and is of reasonable magnitude based on the clinical appearance of the subjects. It is of interest that none of these edematous patients showed thiosulfate spaces in the "excessive" range (over 35% of body weight) often found in observing the radiosodium space or thiocyanate space in sick patients. Similarly it is reassuring that they all manifested increases of an absolute magnitude comparable to the volume of clinical edema (diuresis).

DISCUSSION

I. Characteristics of thiosulfate as an "extracellular" ion

Sodium thiosulfate was first investigated as an antichemical-warfare agent and was found nontoxic even in very large doses, the only untoward effects observed being nausea and vomiting. These appeared when high concentrations were injected rapidly and were considered due to hypertonicity. It was also observed that the ion appeared in the urine at a rate which suggested that its excretion was a function of glomerular filtration. Thereafter it was extensively studied as a renal clearance test and found to have a clearance ratio to inulin and creatinine of 1.0 (both in normals and in renal and cardiac disease states [3–5]). Gilman, Philips, and Koelle (4) suggested and Brun (5) unequivocally asserted that the thiosulfate ion was confined to the extracellular space and not absorbed by the renal tubules. The former (4) calculated volumes of distribution using the amount recovered in the urine while Schwartz, using a constant infusion technique, estimated the space in dogs and man (6).

TABLE VI

Volume of thiosulfate distribution in edematous patients

Patient	Sex	Age	Diagnosis	Body weight	Thiosulfate space	
		yrs.		*kgm.*	*liters*	*% b. wt.*
M. L.	F	37	Lymphedema, etiology unknown	93.2	20.0	21.5
K. R. M.	M	42	Malignant hypertension	68.7	19.8	28.7
L. H.	F	25	Nephrotic syndrome	59.8	14.6	24.5
F. M.	M	74	Congestive heart failure	67.2	16.3	24.3
J. J.	M	70	Chronic nephritis, uremia	96.6	20.7	21.9

Contrasted with inulin which has a large molecular weight of 5,100 and a slow diffusion coefficient of 0.20/cm.²/day, thiosulfate has a molecular weight of only 135 and diffuses rapidly with a diffusion coefficient of 0.68/cm.²/day (7). In this respect it lies between inulin and the halides and light metals whose atomic weights are under 100 and which diffuse very rapidly at a rate of approximately 1.4/cm.²/day (7, 8).

Binding to plasma proteins similar to that which occurs with the thiocyanate ion has been investigated by Kowalski and Rutstein (9). Their *in vitro* studies indicate that no protein binding of thiosulfate takes place.

From the data presented in Table I it is apparent that significant penetration of thiosulfate into erythrocyte water occurs after 70 minutes. If penetration does not occur instantaneously, the extrapolation method used will correct for loss into erythrocyte water.

It is well known that thiosulfate crosses some cell membranes since it cannot be recovered com-

pletely from the urine. In a large series of dogs, Gilman, Philips and Koelle (4) were able to rerover 70–80% in the urine by the time the plasma concentration had fallen below 1.0 mgm.%. In dogs with ligated ureters he observed that the slope of disappearance was linear at 0.05% to 0.10% per minute and represented about 7% of the renal clearance. In this laboratory, 65% of the dose administered to a normal healthy adult male human was recovered by the time the plasma concentration was 1.0 mgm.% and only negligible amounts thereafter. This suggests that recovery in man may be slightly less than in the dog where extremely rapid elimination through the kidneys compared with the volume of distribution reduces the time during which significant metabolism may occur. Gilman and his associates (4) concluded that thiosulfate degradation occurs primarily during and immediately following injection and that after equilibration only slow destruction occurs. One observation offered in support of this postulation was the expanding volume of distribution from approximately 35% of the body weight at 30 minutes to 80% of the body weight at 120 minutes, noted in four dogs and calculated on the basis of the retained dose (the difference between the injected dose and the amount excreted in the urine). These data indicate that metabolism of thiosulfate during the post-equilibrium period must account for the expanding volume of distribution noted. In fact, if one plots their data on semi-logarithmic coordinates and extrapolates to zero time, the thiosulfate space so obtained is approximately 25% of the body weight, which agrees well with the values obtained in our series (24.4% of the body weight, *cf.* Table III) and with those obtained by these authors (4) in the same animals using the Newman formula (10). One is therefore forced to conclude that destruction of thiosulfate is uniform throughout the post-injection period. The discrepancy that remains unexplained is that between the rate of degradation of thiosulfate based on the slow fall in plasma thiosulfate concentration in the anuric dog and the rate of degradation based on the expanding volume of distribution during the post-equilibrium period with intact renal function.

II. The mathematical model

The volume of distribution of a hypothetical substance which could be completely and equally mixed throughout this volume instantly, could be expressed by:

$$V = \frac{A}{P} \qquad (1)$$

V = the volume of distribution,
A = the total substance introduced,
P = the concentration after mixing.

The volume of distribution of any substance which is very rapidly and freely diffusible throughout its volume and which disappears by any route at a rate proportional to its concentration may be determined by plotting its concentration semi-logarithmically against time and extrapolating to its theoretical equilibrium concentration at zero time for substitution in equation (*1*).

$$V = \frac{A}{P \text{ at } t_0}. \qquad (2)$$

That is, the volume of distribution is equal to the amount of material dissipated into the space divided by the concentration which would have occurred had it been instantly distributed throughout the volume at zero-time. The mathematical justification and experimental basis for equation (*2*) is implicit in the derivations of Newman, Bordley, and Winternitz (10) which relates the volume of distribution of a non-metabolized substance to the renal clearance and the time decrement of the natural logarithm of the plasma concentration and in the Schwartz (6) modification which takes into account clearance by all routes.

The primary error involved in applying equation (*2*) is the renal loss incurred at concentrations greater than that accounted for by extrapolation during the period of equilibration (Figure 1). For a rapidly diffusible substance equilibrium time is short enough to make this error insignificant. Employment of such a substance eliminates the necessity for constant infusion or urine collections. It permits the use of a substance lost by extrarenal routes (including metabolism) provided that cell penetration is slow as compared with the rate of distribution in the interstitial fluid. The studies reported in this paper indicate that sodium thiosulfate approaches the requirements for the use of this expression.

Examination of Figure 1 reveals that equilibrium occurs in 10–12 minutes in that the concentration falls logarithmically from this time on and that the

curve may be analyzed into "fast" and "slow" rates, the former probably representing transfer across the capillaries while the latter represents total clearance by all routes. The curve may be described by the general formula (11):

$$C_s = C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}, \qquad (3)$$

where C_s is the concentration at any time t in serum.

C_1 is the concentration obtained by extrapolation of the rapid component of the curve to time zero.

C_2 is the concentration obtained by extrapolation of the slow or equilibrium component of the curve to time zero.

λ_1 is the slope of the rapid component of the curve.

λ_2 is the slope of the equilibrium or slow component of the curve.

Since λ_1 is much faster than λ_2, the requirement of rapid distribution as compared to its total clearance is met. Further evidence indicating rapid equilibration as compared to total clearance is the close agreement for thiosulfate volumes of dilution given by simultaneous arterial-venous sampling. If there were a sizeable gradient across the capillary, one would expect significant arterial-venous differences.

Study was focussed on the equilibrium disappearance rate. The linearity of total clearance as observed by other investigators (3, 4, 6) was confirmed.

The single injection technique described in this paper eliminates the elaborate constant infusion equipment, the long equilibration period, and the addition of a large volume of fluid added to the extracellular compartment. Like the Schwartz technique (6), it does not require urine collections or bladder washouts and obviates the possible errors due to changing urine blank and renal delay time.

Its sole theoretical disadvantage is the possible error due to loss (by all routes) while the concentration of thiosulfate in the plasma is in excess of the extrapolated equilibrium value.

Validity of the single injection technique is dependent on three requirements: (*1*) The extracellular space must remain constant throughout the

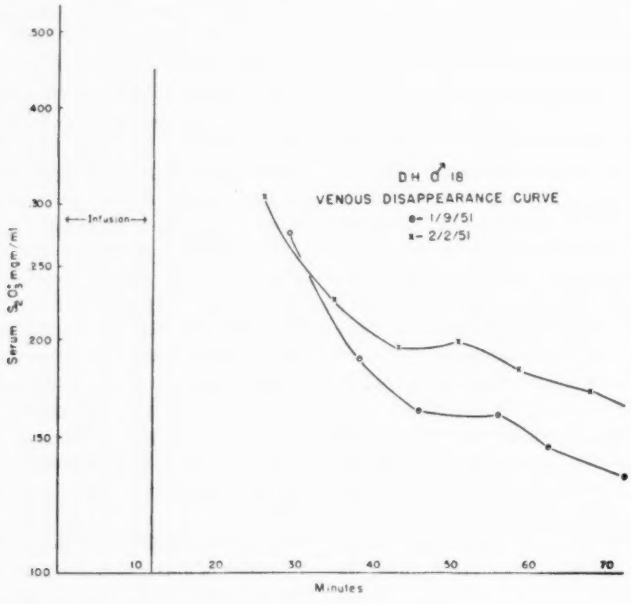

FIG. 4

Subject was a healthy male student, age 18. No history of renal or other disease. 102 ml. 10% $Na_2S_2O_3 \cdot 5H_2O$ were administered by infusion over 11.3 minutes. Samples were drawn from the antecubital veins and serum thiosulfate plotted logarithmically against time.

experiment. The small amount of fluid added by the technique described and the short time required for the measurement tend to insure this. (*2*) There must be uniform and rapid distribution of the substance throughout the space. This appears to occur in about 10 minutes for the thiosulfate ion. (*3*) The substance must be removed from its volume of distribution by all routes at an exponential rate. This has been shown to occur.

The slopes of decrement (in two studies on one subject) in which total clearance rate appears to have been variable are shown in Figure 4. Obviously this curve does not permit extrapolation to a zero-time value and it is anticipated that when such individuals are encountered clinically they will be at once recognized by the characteristics of the curve and no attempt made to estimate the volume of dilution by this method.

III. Evaluation of data and comparison with other techniques

Comparison of simultaneous arterial and venous rates of disappearance shows a consistent lag in the rate of fall of venous values, even though the difference in volume of distribution at zero-time obtained by extrapolation is insignificant, as described above. Brun, Hilden, and Raaschou working with diodrast in dogs (12) point out that the A-V difference is due to the presence in the right ventricle of cleared blood from the renal veins. The much greater difference in dogs is explained by the fact that the ratio of the renal clearance to the volume of distribution in dogs is 70/3 compared with 130/12 in man or almost 2.5 times as great in dogs. Hence a far larger part of the volume of distribution is being cleared in dogs per unit time.

The volumes of distribution of thiosulfate in dogs and man have been determined in this small series to be 24.4% and 16.6% of body weight, respectively. The latter figures were reproducible within 2% of the body weight after a month or more in a steady state. The lower volumes obtained by Schwartz in dogs (17% of the body weight) may be due to a greater fat content in his animals. Gilman, Philips, and Koelle (4) obtained volumes of 22% of the body weight in dogs, agreeing with our values and Schwartz obtained values of 15.7% and 19.5% in two human subjects, agreeing well with our series (Table IV). The volumes are close to those determined with inulin by Gaudino and other investigators (13, 14) of 21–23% for the dog and 13.5–17.5% for man. Turning to the other extracellular dilution indices, chloride, bromide, and sodium are excreted slowly but are known to enter cells to varying degrees and to be concentrated in certain cellular areas (14–18). The sulfate ion is endogenously produced at varying and unpredictable rates and is therefore unsatisfactory (19, 20).

Thiocyanate has been extensively studied as a measure of the extracellular compartment (21, 22). It diffuses rapidly and is slowly excreted permitting long equilibration (22). Its volume of distribution (22–27% of body weight) is similar to that for bromide and sodium which are known to have considerable intracellular fractions and is considerably larger than the inulin space where the large molecule is presumed to be limited to extracellular areas. Protein binding of thiocyanate has been demonstrated by Scheinberg and Kowalski (23). It is known to penetrate the red cell and to be concentrated in saliva and other gastrointestinal secretions (24).

Overman (25) has shown that in febrile states the permeability of the cell membrane to thiocyanate is altered and that its volume of distribution approaches the total body water. Three studies (reported herein), with thiosulfate in febrile patients, resulted in volumes of distribution in the normal range; hence it is probable that no gross changes in cell permeability to thiosulfate occur as a result of fever.

As part of the preliminary evaluation of this technique, five patients with obvious edema were studied. The finding of an increased volume of dilution in all of these patients supports the concepts that by this method an accurate estimate of the extracellular fluid volume can be made.

The final evaluation of this method will depend on the results obtained after wide application by many workers. Further studies on the rates of penetration of thiosulfate into fluid compartments and repeat studies in patients with known losses and/or gains of extracellular fluid are essential.

SUMMARY

A technique for measurement of the extracellular fluid space employing a single brief injection of sodium thiosulfate and collection of several small

blood samples over a short period is presented. This method obviates constant infusion and urine collection. Evidence is presented that sodium thiosulfate is a suitable substance for the relative measurement of the volume of extracellular water.

While there was a consistent lag in the fall of venous concentration behind arterial concentration, no significant difference in the volume of distribution obtained by extrapolation was demonstrated.

The volumes of distribution of sodium thiosulfate in seven dogs and six humans averaged 3 liters or 24.4% of body weight and 12.2 liters or 16.6% of body weight, respectively.

Thiosulfate space in patients with fevers of 102–103.5° F. were in the normal range and in patients with edema were well above the normal range. The volume of distribution of thiosulfate in edematous patients was compatible with known increase in weight and clinical estimates of edema fluid.

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SINGLE INJECTION TECHNIQUE FOR MEASUREMENT OF THE VOLUME OF DISTRIBUTION OF THIOSULFATE

APPENDIX I

Preparation—(under sterile conditions): Twelve 1.0 gm. ampules of $Na_2S_2O_3 \cdot 5H_2O$* are dissolved in a sterile beaker with 120 ml. sterile pyrogen-free water and thoroughly mixed. Twenty ml. are withdrawn for the determination of the density of the solution and titration of the concentration of thiosulfate. The remainder is used to fill a calibrated 100 ml. syringe.

Administration: An infusion is started with saline or 5% glucose using an open-top set. When the chamber of the infusion set is empty, the thiosulfate solution is quickly introduced from the calibrated syringe.

A stop-watch is started when the thiosulfate solution reaches the needle and this is considered zero-time for calculations.

* Sulfactol, Winthrop-Stearns, Inc., N. Y. C.

Flow should be regulated, before addition of the measured thiosulfate, to approximate seven to 10 ml. per minute so that the infusion will last ten to 15 minutes. Upon completion of the infusion, the time should be noted and the set washed through with 20–30 ml. of normal saline or 5% dextrose solution three times.

Samples: Blood samples should be 1–2 ml. in volume. The blank titration is considerable (about 0.04 mgm./ml.). A blank should be drawn at the time the needle is inserted for the infusion. Venous samples must be drawn without stasis and midtimes recorded. Between samples the arm should be kept under a blanket. A minimum of four samples evenly spaced over 20–70 minutes after the start of infusion should be drawn.

APPENDIX II

Chemical Method

I. Reagents

1% Na_2WO_4
$\frac{N}{15}$ H_2SO_4
0.001 N (exact) KIO_3
0.0005 N $Na_2S_2O_3$
2 N HCl
10% KI
1% soluble starch.

Comment: The KIO_3 must be made up precisely and is stable. The $Na_2S_2O_3$ tends to deteriorate and must be checked at the beginning and end of each run. Deterioration is due to sulfur-metabolizing bacteria and may be slowed by using sterile distilled water and adding 0.1 gm. Na_2CO_3 per liter. KI should be prepared freshly with each set of determinations and kept in a brown or opaque container.

The starch solution is prepared by boiling. It is then centrifuged and decanted, and can be stored on ice for a few days.

II. Procedure

A. Protein-free filtrates

Samples are centrifuged as soon as possible and clear serum must be obtained.

To 2 ml. $\frac{N}{15}$ H_2SO_4 in 16 ml. H_2O, add 0.2 ml. serum slowly with agitation.
Then add 2.0 ml. 1.0% Na_2WO_4 slowly with agitation.
Permit to stand 10 minutes, centrifuge, decant, and save clear supernatant fluid.

B. Infused solution

The weight of the infused thiosulfate solution contained in a calibrated 10 ml. pipette is determined on a chemical balance and the density calculated.

Quadruplicate 0.10 ml. aliquots are then weighed to 0.1 mgm. and diluted in liter volumetric flasks with distilled water and 8 ml. aliquots of both dilutions titrated as below.

*C. Titration**

To 5 ml. 0.001 N KIO_3, add 8 ml. protein-free filtrate and 1 ml. 2 N HCl.

Let stand at least five minutes and not more than 10 minutes.

Then add

1 ml. 10% KI and titrate immediately with 0.0005 N $Na_2S_2O_3$ using 2 to 3 drops 1% starch indicator.

Comment: Duplicate 8 ml. aliquots of the diluted infused solutions, serum blank, serum samples, and a reagent blank are titrated. Starch should not be added until the yellow iodine color has almost disappeared.

III. Calculations

$$A = W_I \times C_I, \qquad (1)$$

where: A = mgm. of $S_2O_3^=$ administered.

W_I = volume in ml. delivered by the calibrated 100 ml. syringe

C_I = concentration in mgm./ml. of the infused thiosulfate solution.

$$C_I = (t_s - t_u)\left(\frac{0.158}{8}\right)\left(\frac{5}{t_s}\right)\left(\frac{D.F.}{8}\right) \qquad (2)\dagger$$

$$C_p = (t_B - t_u)\left(\frac{0.158}{8}\right)\left(\frac{5}{t_s}\right)\left(\frac{100}{8}\right), \qquad (3)\dagger$$

where: C_p = concentration in mgm./ml. of thiosulfate in serum.

t_s = ml. of 0.0005 N $Na_2S_2O_3$ required to titrate 5 ml. of 0.001 N KIO_3 (reagent blank).

t_u = ml. of 0.0005 N $Na_2S_2O_3$ required to titrate unknowns other than serum blank.

t_B = ml. of 0.0005 N $Na_2S_2O_3$ required to titrate serum blank.

D.F. = dilution factor, obtained by dividing 1000 by the weight of the aliquot and this divided by the density of the infused solution.

$$V = \frac{A}{C_{Po}}, \qquad (4)$$

where: V = volume of distribution for thiosulfate

C_{Po} = concentration in mgm./ml. of thiosulfate in serum at zero-time. This is obtained by plotting on semi-log coordinates the concentrations of thiosulfate in the serum samples against the mid-point of the time at which they were drawn and extrapolating to zero-time.

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* All glassware must be extremely clean and oxidizing agents must be avoided.

† For derivation of formulas 2 and 3 see Newman, Gilman, and Philips (3).

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THE ELECTROLYTE CONTENT OF SKELETAL MUSCLE IN CONGESTIVE HEART FAILURE; A COMPARISON OF RESULTS WITH INULIN AND CHLORIDE AS REFERENCE STANDARDS FOR EXTRACELLULAR WATER [1]

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In estimating shifts of electrolytes between intracellular and extracellular fluid compartments from metabolic balance data, it is necessary to assume *1*) either an initial or final volume of extracellular water, and *2*) that the balance of chloride reflects changes in extracellular chloride only. Similarly, in calculating intracellular electrolyte concentrations from tissue analyses, it is necessary to assume that the chloride present in tissue is freely diffusible and is derived only from the extracellular fluid space. Recent studies (1–4) have shown, however, that the latter premise may not be entirely correct, and that a significant moiety of tissue chloride may be intracellular. Yannet and Darrow (5) have subtracted 1 mM. of chloride from the total tissue chloride per 100 grams of fat-free solids to correct for this nonextracellular chloride in muscle. This correction averages about 15 per cent. Since the volume of distribution of inulin is smaller than that of chloride or other electrolytes, it has been suggested that inulin is confined to the extracellular compartment. Therefore, in an effort to obtain a more precise measurement of the extracellular fluid of muscle, inulin was infused into experimental subjects until uniform distribution had been achieved. Then muscle was obtained by biopsy and analyzed for inulin and electrolyte content. As will be seen from the results obtained, intrinsic analytical variations may occasionally conceal any real difference between the chloride and inulin "spaces."

Metabolic balance data (6–8) on patients recovering from congestive heart failure show in most instances a retention of potassium in excess of nitrogen. This has been interpreted as a replacement of potassium lost during the development of failure. Cardiacs in whom edema is produced by the addition of salt (9) and/or the withdrawal of digitalis (9, 10) may either be in balance or have negative or positive potassium balances; thus a negative potassium balance is not a necessary concomitant of congestive heart failure. These indirect findings are substantiated in part by our direct chemical analyses of muscle of patients in heart failure.

METHODS

Six patients in chronic congestive heart failure, who had varying amounts of pitting peripheral edema but, with one exception (G. M.) no demonstrable pleural effusion or ascites, and four non-cardiac control subjects were studied. The cardiacs received no digitalis or mercurials for at least two weeks prior to study with one exception, I. F., who developed acute pulmonary congestion and who received digoxin about 36 hours prior to muscle biopsy. The controls and several of the cardiacs were given a regular hospital diet. Some of the cardiacs were on salt poor diets containing 5 to 15 mEq. of sodium and about 70 mEq. of potassium, to which 5 to 10 grams of sodium chloride were added daily for variable periods until the signs and symptoms of congestive heart failure were manifest.

While in the post-absorptive state with water permitted *ad libitum*, the control subjects were given a constant infusion of 10 per cent inulin in 0.45 per cent saline and 2.5 per cent glucose for at least six hours, at the end of which time it has been shown (11) that inulin has equilibrated in the extracellular compartment. In the cardiacs, however, the equilibration time was prolonged to 30 hours.[2] The priming and sustaining infusions were given at a rate calculated to result in an extracellular in-

[1] This study was aided in part by a grant from the National Heart Institute, the United States Public Health Service, and the Martha M. Hall Foundation.

[2] This time was determined by expediency and by the fact that interstitial and plasma inulin concentrations fell within the analytic error. Constancy of plasma concentration does not insure extracellular compartment saturation.

During this period about 1,800 ml. of 10 per cent inulin in 0.3 per cent saline and 3.3 per cent glucose were infused. There was no significant change in extracellular fluid volume since most of the solution was excreted. A more concentrated inulin solution suitable for infusion was not available.

ulin concentration of about 1 mg./ml. in the cardiacs and about 2 mg./ml. in the controls. There were no untoward effects with these high inulin concentrations in any of the subjects. While the inulin infusion was continuing and presumably equilibration had been achieved, a biopsy of the gastrocnemius muscle was taken under spinal anesthesia. Simultaneously, blood was sampled from the femoral artery. In the cardiacs, edema fluid was also obtained from the leg from which muscle was removed. The inulin in plasma and edema fluid was analyzed by the resorcinol method of Roe, Epstein, and Goldstein (12) and the inulin in muscle was analyzed by a modified resorcinol method developed by the authors (13). The inulin volume of distribution was determined according to the technic of Gaudino, Schwartz, and Levitt (14) and Schwartz, Schachter, and Freinkel (11).

The muscle was freed from visible blood, connective tissue, and fat and treated in the following manner: *1*) Samples of 1 to 2 grams were analyzed for inulin. *2*) The blood content of the muscle was estimated on triplicate 0.3 to 0.5 gram samples taken from different sites and extracted with 3 to 5 ml. of normal saline for about one hour at a reduced temperature with occasional stirring. The red cells in the saline extract were counted in a chamber and its equivalent as whole blood was calculated from the red cell content of peripheral blood. Neither spectrometric determination of the blood in the muscle, as suggested by Lowry and Hastings (15), nor the method of Fanelli (16) for determining blood hemoglobin in the presence of muscle myoglobin was employed because the method of counting red cells was more convenient. *3*) The remaining muscle was minced and the wet-dry weight determined. *4*) The dried contents were then ground to a fine powder in an agate mortar and aliquots taken for the following analyses: total lipids by extraction with alcohol-ether in the Soxhlet apparatus, evaporating to dryness and weighing the residue after re-extraction with petroleum ether; chloride and phosphorus by mixing the dried solids with an excess of chloride-free sodium carbonate and ashing in a muffle at 450° C. The alkaline ashed material was dissolved in sulfuric acid and an aliquot taken for phosphorus determination according to Fiske and SubbaRow (17) and for chloride analysis according to Van Slyke and Hiller (18); sodium, potassium and magnesium on a separate aliquot ashed overnight at 450° C. in a separate furnace. Sodium was determined gravimetrically as the uranyl zinc acetate salt (19), potassium was determined titrametrically after precipitation as the iodoplatinate (20, 21), and magnesium was determined colorimetrically as phosphate in magnesium ammonium phosphate after the removal of calcium as the oxalate (22); nitrogen by micro-Kjeldahl digestion, steam distillation, and titration.

All the muscle analyses were done on separate duplicate and triplicate aliquots and showed the following deviation from the mean: potassium, ± 0.8 per cent with a range of 0 to 2.2 per cent; chloride, ± 1.1 per cent with a range of 0 to 4.1 per cent; sodium, ± 1.9 per cent with a range of 0.2 to 4.4 per cent; fat, ± 2.5 per cent with a range of 0 to 5.2 per cent; inulin, ± 5.3 per cent with a range of 0.5 to 9.0 per cent.

The bicarbonate content of serum was determined manometrically (23). The analytic methods described above for muscle electrolytes were employed also for serum electrolytes.

Sodium and potassium in whole blood were determined with a Perkin-Elmer flame photometer, model 52A, after the red cells were hemolyzed with distilled water. Whole blood chloride was determined on a picric acid filtrate, as described by Van Slyke and Hiller (18).

Calculations

The concentration of electrolytes in extracellular water was calculated from their concentration in serum corrected for serum water and an average Donnan equilibrium factor of 0.96. The muscle analyses were corrected for the variable amount of fat and blood present in the specimen sampled and are expressed as *fat-free, blood-free solids.* Darrow (5, 24) has suggested the use of fat-free solids as a reference base because these are practically all intracellular and are independent of the extracellular fluid volume. However, variables in the intracellular solid composition such as glycogen may at times make this reference standard inexact for describing muscle composition.

For comparative purposes, the *extracellular water* $(H_2O)_e$ per 100 grams fat-free, blood-free solids was calculated using three different standards for measuring extracellular water as follows:

1. $\dfrac{\text{mg. inulin per 100 grams solids}}{\text{mg. inulin per gram serum water}} = \text{grams } (H_2O)_e$

2. $\dfrac{\text{mM.Cl per 100 grams solids}}{\text{mM.Cl per gram extracellular water}} = \text{grams } (H_2O)_e$

3. $\dfrac{\text{mM.Cl per 100 grams solids} - 1}{\text{mM.Cl per gram extracellular water}} = \text{grams } (H_2O)_e,$

as suggested by Yannet and Darrow who reported that 1 mM. of chloride per 100 grams fat-free muscle solids is not freely diffusible (5).

The *extracellular electrolyte content* of the muscle is the product of the extracellular water and the concentration in that water.

Intracellular water was calculated as the difference between total water and extracellular water. Similarly, the *intracellular electrolyte content* was calculated as the difference between the total electrolyte content of the muscle and the extracellular value; they are expressed as concentrations per kgm. intracellular water (Table II).

No attempt was made either to determine the collagen and elastin content of the muscle or to differentiate nonprotein nitrogen from protein nitrogen in muscle.

The total body volume of distribution of inulin was calculated according to the technic of Gaudino, Schwartz and Levitt (14) and Schwartz, Schachter, and Freinkel (11), except that no urine correction blank was applied because the inulin equilibrium concentration in the extracellular water was about ten times the concentration usually achieved.

RESULTS

The analytical results and the derived calculations are presented in Tables I, II, and III. An orientation on the interpretation of muscle analyses is given by Darrow (24).

Extracellular water. In the four non-cardiac subjects the extracellular water in the muscle was on the average 18 per cent greater when measured with chloride than with inulin. In patients A. N. and W. Z., the chloride "space" in the muscle was as much as 31 per cent and 41 per cent greater than with inulin. If one corrects for non-diffusible chloride as suggested by Darrow, the extracellular water value on the average was only 4 per cent greater than when calculated with inulin. The range, however, was 15 per cent less to 23 per cent greater than the inulin value (Table III). In absolute terms, the amount of extracellular water associated with 100 grams fat-free, blood-free solids varied from 39 to 59 grams, 55 to 59 grams, and 47 to 50 grams estimated from inulin, chloride, and chloride corrected for the non-diffusible moiety, respectively (Table II). On a percentage basis, the extracellular water averaged 9.8 per cent, 11.6 per cent and 10.0 per cent of the wet tissue, respectively, employing the above standards for extracellular water. In the three cardiacs, I. F., Y. J., and F. P., in whom, we believe, distribution equilibrium in the extracellular water of the muscle had been achieved with inulin as shown by the equality of the inulin concentration in the plasma and in the edema fluid in the leg from which the muscle was taken, the extracellular water associated with 100 grams fat-free solids was 96, 98, and 112 grams. The corresponding values obtained with chloride in these three cardiacs were 98, 92, and 100 grams of interfiber water per 100 grams fat-free solids. Similarly, applying the correction factor for non-diffusible chloride, the values were 89, 85, and 91 grams of extracellular water per 100 grams fat-free solids. On a wet basis, the extracellular water in the muscle of the cardiacs was about twice that of the controls. The *volume of distribution* of inulin averaged 14.1 per cent of the body weight in the controls and was 27.8 per cent, 36.6 per cent and 24.5 per cent of the body weight in the three cardiacs, I. F., Y. J., and F. P., respectively.

Intracellular water. The value calculated for

TABLE I

Summary of analyzed data

A. Patients without congestive failure

Patient*	Age	Diagnosis†	Serum concentration per liter						Muscle composition								
									Per 100 grams fat-free, blood-free solids								Per 100 grams tissue
			H_2O	Cl	CO_2	Na	K	Mg	H_2O	Cl	Na	K	Mg	N	P	K/N	Fat-free solids
			grams	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*grams*	*mM.*	*mM.*	*mM.*	*mM.*	*grams*	*mM.*	*mM./gram*	*grams*
A. W.	38	Cerebellar degeneration	921	105.4	21.5	137.3	4.4	0.84	362	7.0	11.4	48.1	3.88	15.6	31.4	3.08	21.0
A. N.	27	Normal	917	106.5	25.4	134.0	4.1	0.96	362	7.1	11.9	48.1		15.7	30.8	3.06	20.4
W. J.	50	Strabismus	917	110.0	26.4	144.0	4.0	0.88	340	6.9	11.5	43.5	3.65	14.3	29.8	3.04	20.1
W. Z.	42	Cataract	912	105.7	28.1	139.5	3.7	0.94	359	6.6	11.6	46.8	4.20	15.4	31.2	3.04	20.0
B. Patients with congestive failure																	
M. M.	60	ASHD	917	112.6	16.2	138.2	5.8		435	17.0	25.5	41.4	4.20	15.6	30.1	2.66	17.5
I. F.	47	RHD	922	105.8	20.6	127.0	3.3		405	11.7	18.2	45.0	4.35	15.1	29.2	2.98	18.2
Y. J.	33	SHD	929	109.8	19.7	137.0	4.4	0.72	383	11.4	16.7	42.4	5.00	14.2	28.6	2.99	20.1
F. P.	25	RHD	920	107.0	22.9	140.0	3.2	0.74	433	12.1	20.3	47.3	4.95	14.6	31.0	3.24	17.7
G. M.	41	RHD and constrict. pericard.	941	80.5	42.0	130.1	4.5	0.63	553	17.6	31.0	51.0	4.38	14.9	31.0	3.32	13.7
J. A.	44	HHD	917	107.7	19.4	129.9	4.0	0.93	446	12.6	18.6	42.0	5.10	15.6	31.1	2.69	17.2

* All patients except I. F. and F. P. were males.

† ASHD = Arteriosclerotic heart disease.
RHD = Rheumatic heart disease.
SHD = Syphilitic heart disease.
HHD = Hypertensive heart disease.

TABLE II

Calculated values of intracellular electrolytes based on the various methods for measuring extracellular water

A. Controls

Patient	H_2O per 100 grams fat-free solids						Intracellular concentration per kgm. H_2O								
	Extracellular			Intracellular			Na			K			Mg†	N†	P†
	Inulin	Chloride	Chloride-1	Inulin	Chloride	Chloride-1	Inulin	Chloride	Chloride-1	Inulin	Chloride	Chloride-1			
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*grams*	*mM.*
A. W.	59	59	50	303	303	312	10.0	10.0	13.5	158	158	153	12.5	51	102
A. N.	45	59	50	317	303	312	17.6	12.3	15.7	151	158	153		50	99
W. J.	48	55	47	292	285	293	14.4	12.2	15.0	148	152	148	12.4	49	103
W. Z.	39	55	48	320	294	311	18.4	11.2	14.5	145	158	150	13.5	50	101
B. Cardiacs															
M. M.		133	125		302	310		20.8	24.2		135	131		51	98
I. F.	96	98	89	309	307	316	17.8	17.3	20.2	145	145	141		49	94
Y. J.	98	92	85	285	291	298	10.2	12.4	15.8	147	145	141	17.0	49	98
F. P.	112	100	91	321	333	342	12.2	17.7	20.5	146	141	137	14.6	44	94
G. M.*		195	185		358	368		15.1	17.4		140	136	11.8	41	86
J. A.*		102	94		344	352		14.2	16.5		121	118	14.4	45	89

* Inulin was not infused in patients G. M. and J. A. because the presence of massive edema, pleural effusion, or ascites in these patients precluded the attainment of equilibrium distribution within a reasonable period of time.

† These concentrations are calculated with an average value for intracellular water.

this datum as well as the value for intracellular electrolyte concentration is dependent primarily upon the accuracy with which the extracellular water can be measured. Since there is no single absolute unit of reference for extracellular water, we have calculated it with the three frequently used reference standards. It will be seen (Table II) that in the normal subjects when the values for the extracellular water obtained with inulin are subtracted from the total water, the intracellular water varied from 292 to 320 grams per 100 grams fat-free solids. The maximum variation in intracellular water calculated with the several extracellular water values occurred in W. Z. and amounted to 8 per cent. There was no increase in intracellular water in the three cardiacs who were equilibrated with inulin. If one considers the six cardiacs as a group, the average increase of 7 per cent in intracellular water is not significant ($P > .05$) as compared to the four normals. It appears that the increased water content of the skeletal muscle in cardiacs is due to extracellular fluid accumulation, and not to any plethora of intracellular water.

TABLE III

Extracellular water ratio and volume of distribution

A. Controls

Patient	Extracellular H_2O ratio		Volume of distribution			
			Wet muscle			Body weight
	Chloride / Inulin	Chloride-1 / Inulin	Inulin	Chloride	Chloride-1	Inulin
			per cent	*per cent*	*per cent*	*per cent*
A. W.	1.00	0.85	12.4	12.4	10.5	15.7
A. N.	1.31	1.11	9.2	12.0	10.2	12.4
W. J.	1.15	0.98	9.7	11.1	9.5	14.3
W. Z.	1.41	1.23	7.8	11.0	9.7	14.0
B. Cardiacs						
M. M.				23.3	21.9	
I. F.	1.02	0.93	17.5	17.8	16.3	27.8
Y. J.	0.94	0.87	19.7	18.5	17.2	36.6
F. P.	0.89	0.81	19.8	17.6	16.1	24.5
G. M.				26.7	25.4	
J. A.				17.6	16.1	

The *sodium* content of the muscle averaged 11.6 ± 0.21 mM. per 100 grams fat-free solids in the four non-cardiacs and 21.7 ± 5.55 mM. per 100 grams fat-free solids in the cardiacs with variable edema. The *chloride* content averaged 6.9 ± 0.21 mM. per 100 grams fat-free solids in the normals, and 13.8 ± 2.77 mM. per 100 grams fat-free solids in the cardiacs. The Na/Cl ratio in the two groups, 1.68 in the normals and 1.58 in the cardiacs, was essentially the same. The slightly lower ratio in the cardiacs is not surprising since in this

group there were increased amounts of extracellular water derived from serum with a Na/Cl ratio of 1.2. Had there been a *selective* intracellular gain of sodium in the cardiacs there should have been either an increase [3] in this ratio in the muscle or a decreased interfiber Na/Cl ratio. Moreover, the intracellular sodium concentration calculated with inulin as the reference base for extracellular water varied to the same extent in both groups. In the cardiacs, the sodium concentration varied from 10.2 mM. to 17.8 mM. per kgm. intracellular water and in the normals, the range was from 10.0 mM. to 18.4 mM. of sodium per kgm. intracellular water. If the assumption is made that chloride is exclusively confined to the extracellular space, then on the average the cardiacs had a 42 per cent increase in intracellular sodium concentration as compared to normals. Similarly, with a fixed correction for non-extracellular chloride according to Darrow, the cardiacs had on the average a 30 per cent increase in intracellular sodium as compared to the four control subjects.

The *potassium* content of the muscle averaged 46.6 ± 2.10 mM. per 100 grams fat-free solids in the normals as compared to 44.9 ± 3.75 mM. per 100 grams fat-free solids in the cardiacs (Table I). This difference is not significant. Only a small moiety, 0.5 to 1.7 per cent of the total muscle potassium of all the subjects, is contained in the extracellular water; therefore, the intracellular *concentration* of potassium is determined to a greater extent by the amount of intracellular water than by the amount of potassium in the extracellular compartment. It will be seen (Table II) that in the normals the intracellular potassium concentration averaged 151 mM., 157 mM., and 151 mM. per kgm. water as calculated from inulin, chloride, or chloride-1, respectively, as the standards for extracellular water. In the cardiacs, the corresponding average values were 146 mM., 138 mM., and 134 mM. potassium per kgm. intracellular water. The difference in the average intracellular potassium concentration in the two groups is about 10 per cent.[4]

The *potassium/nitrogen* ratio in the muscle of the four non-cardiacs varied slightly from 3.04 to 3.08 mM. potassium per gram nitrogen, whereas in the cardiacs there was a greater variation of from 2.66 to 3.32 mM. potassium per gram nitrogen. The difference of the means in the two groups is not significant. Of interest are the results obtained in M. M., who had a second biopsy on the opposite leg after a 13.3 kgm. weight loss and disappearance of his peripheral edema. In failure, the K/N ratio (Table I) was 41.4/15.6 or 2.66 mM. potassium per gram nitrogen, and when compensation was restored, the K/N ratio was 48.1/15.4 or 3.12 mM. potassium per gram nitrogen.

The *magnesium, nitrogen,* and *phosphorus* content (Table I) of the muscle in the cardiacs and controls did not differ significantly. The figures in Table II are based on an *average* intracellular water content. It will be seen that on the average there was 7 per cent less nitrogen and 8 per cent less phosphorus in the cardiac group than in the control patients.

When the intracellular concentrations of K, Mg, and Na are added, the total cation concentration per kgm. intracellular water is equivalent to 168 mM. in the cardiacs and 179 mM. in the non-cardiacs; the difference is chiefly attributable to the lowered potassium concentration in the cardiac.

Except for the lower total CO_2 in the cardiacs, the serum electrolytes in the two groups were essentially similar. Patient G. M. always had an elevated CO_2 and decreased chloride. The blood pH was 7.40. We attributed these serum findings to a primary CO_2 retention secondary to pulmonary pathology, although at autopsy there was only a moderate interstitial pulmonary fibrosis.

DISCUSSION

In the present study an attempt has been made to limit the number of variables that may influence the results. In many instances the blood content of excised human muscle is not negligible. On the average there was 1 per cent blood in the muscle samples which if uncorrected would increase the extracellular water by about 5 per cent

[3] The increase in intracellular sodium is a small fraction of the total sodium and may not be readily apparent in the muscle Na/Cl ratio. In the cardiac a 10 per cent increase in the amount of intracellular sodium will result only in a 2 per cent increase in the muscle Na/Cl ratio.

[4] It should be noted that although the increase in intracellular water found in the cardiacs is not significant, on the average it affects a 10 per cent reduction in the intracellular potassium concentration. Derived values may be magnified at times by minor differences in analyzed data.

when chloride is used to estimate this compartment. This correction was of the same order of magnitude in several patients who had high inulin blood levels. The blood contamination error becomes even more significant when the chloride content of muscle is corrected for the non-diffusible moiety. This consideration may not apply to studies in which animals have been exsanguinated and the muscle chilled prior to removal (15).

Certain factors must be considered in relation to the patients used in this study. The electrolyte intake of the subjects, while not rigidly controlled, were sufficiently similar for about 14 days prior to the study probably to preclude any differential influence on the electrolyte content of the muscle. The role of restricted protein intake for protracted periods in several of the cardiacs is unknown. A reduced potassium intake can diminish the potassium content of muscle as shown by Heppel (25) and later by Conway and Hingerty (26) who were able to replace about 25 per cent of the muscle potassium with sodium when rats were placed on a low potassium intake, equivalent to about 0.025 mM. of potassium per 70 gram rat daily for about 26 days. This consideration may not apply to the cardiacs just prior to study, but the influence of possible low potassium intake, mercurials, and digitalis for prolonged periods on the potassium content of muscle cannot be evaluated at present.

Although the etiology of the heart disease varied, all the cardiacs had fixed congestive heart failure since the edema rapidly accumulated if sodium was not rigidly restricted.

The validity of any conclusions to be drawn from the present study is dependent upon the accuracy with which the extracellular water in muscle can be determined. From the present data one cannot select the true reference standard for measuring extracellular water. Levitt and Gaudino (27) have presented evidence, albeit inferential, that the equilibrium volume of distribution of inulin approximates more closely the extracellular fluid compartment than does chloride, thiocyanate, or mannitol. It is for this reason that we have chosen inulin as a reference base for extracellular water. The chief disadvantage in the use of inulin in the edematous cardiac is the difficulty in achieving equilibrium distribution throughout all the recesses of the extracellular compartment within a reasonable period of time (11). In the absence of pleural effusion or ascites, however, equilibrium has been achieved in 30 hours as demonstrated by equality of concentration, within experimental error, in the plasma water and edema fluid. Another criticism of the use of inulin is that the accuracy of its determination in muscle is less than that of chloride. The latter can be determined with an accuracy of about 1 per cent, whereas that of inulin averages about 5 per cent. Some of the variation in the chloride/inulin ratio may be due to these factors.

If inulin is *limited* to extracellular distribution, the average chloride/inulin ratio of 1.18 in the non-cardiacs suggests that a significant portion of muscle chloride is normally non-diffusible and presumably non-extracellular in distribution. This confirms the findings of Harrison, Darrow, and Yannet of a significant moiety of non-diffusible chloride in muscle (4). Whether this is a constant fraction of the total chloride present under normal and pathological conditions and equivalent to 1 mM. per 100 grams fat-free solids as suggested by Darrow's data in cats, is not evident from the present study. The chloride/inulin ratio of 0.95 in the three cardiacs, on the other hand, may be explained by: *1*) A simple expansion of the extracellular volume, since under such conditions a given amount of non-diffusible chloride is a *smaller* fraction of the total chloride; *2*) an actual shift of chloride out of the cells. Under the influence of increased adrenocortical activity it has been reported (28) that chloride shifts into the inulin space. These shifts, however, are of a transient nature and may not apply to a chronic state such as congestive heart failure, although there are data (9, 29) suggestive of hyperadrenocorticism in heart failure. It is not known, though, whether this pertains to the cardiacs in the present study since no attempt was made to obtain evidence for excessive adrenal cortical stimulation.

Conclusions drawn from metabolic balance data based on the premise that the chloride changes are all extracellular should be re-examined in the light of recent data (1–4) including the present study which suggests that chloride may also be intracellular. Elkinton, Squires, and Crosley (30) have called attention recently to the fact that at times absurd values are obtained when chloride is used for calculating extracellular volumes and that an additional reference base such as the volume of distribution of inulin should be employed. The

present data on the volume of distribution of inulin agree with those previously published by Gaudino, Schwartz and Levitt (14) and by Schwartz, Schachter, and Freinkel (11). It is also of interest to note that the inulin "space" determined by immersing frog sartorius muscle in an inulin-Barkan solution (31) was 9.6 ml. per 100 grams muscle and similarly in the present *in vivo* study it was 9.8 ml. per 100 grams muscle; the variation was the same in both studies.

The earlier work of Harrison and co-workers (32–34) and more recently of Alexander and associates (35), reported a decreased potassium content of skeletal muscle in heart failure. The analytical results were not expressed on a fat-free basis and are not entirely comparable with the present data. In the present study, if inulin is accepted as the true reference standard for measuring extracellular water and if equilibrium distribution has been achieved, then the intracellular potassium content of the three cardiacs, I. F., Y. J., and F. P., does not differ from the normals.[5] More data similar to that obtained in M. M. are required before it is concluded that there is a decreased potassium content in the skeletal muscle of congestive failure patients. In patient M. M. there was a distinct increase in the potassium content of the muscle as heart failure waned.

In one month the intracellular potassium content of muscle in a cardiac with a daily negative external potassium balance of 10 mEq. will decrease about 10 per cent. This is equivalent to the decrease observed in the present series of cardiacs if all the values for intracellular potassium are averaged. The assumption is made that the loss is entirely from the skeletal muscle mass which in a 70 kgm. man contains about 2,800 mEq. of potassium. The liver contains only about 4 per cent and the blood about 8 per cent as much potassium as muscle. It therefore appears very unlikely that liver and blood are the major sources of the potassium that is lost when the organism is in chronic negative potassium balance.

The accuracy of the determination of the intracellular sodium will depend largely on the determination of the extracellular value for this cation because it is predominantly outside the cell. It is for this reason that large variations in intracellular sodium concentration are obtained in the same sample depending upon what standard measures extracellular water. In biopsies from the rectus abdominis muscle, Mudge and Vislocky (36), using chloride as the reference standard, obtained values of 2, 8, and 14 mM. of sodium per liter intracellular water in three control patients.

What has emerged from the present studies is the *uncertainty of the interpretation of data relating to intracellular electrolyte concentrations,* determined either directly by tissue analyses or indirectly by metabolic balance studies or isotopic dilution techniques, *unless the extracellular compartment is accurately measured.* Inherent difficulties in methodology may at times obscure any real difference between the inulin and chloride "spaces," and the variation in the chloride/inulin ratio may be such that calculated small differences in intracellular electrolyte concentration may be more apparent than real. If future work proves that the equilibrium volume of distribution of inulin is the correct reference standard, there may be no difference in the intracellular electrolyte composition of patients in congestive heart failure as compared to normal. However, if some modification of the "chloride space" turns out to be the reference base, the cardiac in failure may have a decreased intracellular potassium and an increased sodium concentration, but the exchange between potassium and sodium is not equal.

Yannet and Darrow (5) previously have called attention to the fact that shifts of water are not determined solely by changes in extracellular electrolyte concentration. More recently Opie and Rothbard (37) have concluded that movement of water in striated muscle removed from the body occurs not only under conditions of osmotic interchange but also under conditions which have no relation to electrolyte concentrations. In the dog, Gaudino and Levitt (38) have shown that DCA causes a decrease in intracellular fluid volume and an expansion of the extracellular space. Similar findings are reported in man by Levitt and Bader (28) with cortisone and ACTH. In man and dog the effects are transient. Robinson (39) has concluded from experiments on rat kidney that mammalian cells are not in osmotic equilibrium with their environment and that "the osmotic pressure of the cell fluids is normally 50 to 100 per

[5] It should be noted that an apparent increase in the amount of extracellular water will result in an apparent increase in the *concentration* of intracellular potassium.

cent greater than that of the extracellular fluids." Whether this applies to other tissues as well remains to be determined. While one cannot assess total osmotic pressure from cation concentration alone, it is of interest to note that the intracellular cation concentration in the muscle of the cardiacs and controls was significantly higher than the interstitial fluid. The hypothesis of inactive cell base (40) remains to be proved.

We are not in a position to corroborate with the present limited data the earlier report by Laszlo (41) that the phosphate content of skeletal muscle is significantly reduced in heart failure.

SUMMARY AND CONCLUSIONS

Biopsies of the gastrocnemius muscle were taken in seven patients, four non-cardiacs and three cardiacs with congestive heart failure, after a constant infusion of inulin had been administered for 30 hours in the cardiacs and six hours in the controls. In three additional cardiacs biopsies were taken without prior inulin infusion. On the premise that equilibrium distribution of inulin had been achieved, it was found that in normal patients 48 grams of extracellular water were associated with 100 grams of fat-free muscle solids; using "chloride space" as the reference standard, this value for extracellular water was 18 per cent higher. When a constant correction for non-diffusible chloride was applied, equivalent to 1 mM. of chloride per 100 grams of fat-free solids, the extracellular water content was 4 per cent greater than with inulin. The range, however, was wide. In the three patients with heart failure the chloride/inulin ratio was 0.95 and the chloride-1/inulin ratio was 0.87.

The true value for extracellular water is not evident from the present study. If one accepts the "chloride space" or a "corrected chloride space" as the reference standard for extracellular water, it can be shown that the cardiac with fixed heart failure has a loss of intracellular potassium and a gain of intracellular sodium. With inulin as a standard, there is no change in the intracellular electrolyte composition in heart failure from the normal.

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DISTURBANCES OF IONIC EQUILIBRIUM OF INTRACELLULAR AND EXTRACELLULAR ELECTROLYTES IN PATIENTS WITH TUBERCULOUS MENINGITIS[1]

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Diminished concentrations of chloride and sodium in plasma are frequently found in patients with acute bacterial infections (1). This type of deviation from the normal electrolyte pattern is also encountered in an occasional patient with pulmonary tuberculosis and has received special attention because of the problem of differential diagnosis of Addison's disease (2–5). By the use of methods of assessment of adrenocortical function which do not depend upon determinations of serum electrolytes it has been shown that hypochloremia and hyponatremia may be present in patients with tuberculosis without evidences of adrenal insufficiency (5).

Reductions of the serum chloride and sodium values are consistently found in children with tuberculous meningitis. In the past this observation has not received much attention because of the progressive and uniformly fatal course of this disease, and the abnormalities of electrolyte concentration were usually attributed to lack of salt intake plus losses in vomitus. During the past five years the prognosis and course of tuberculous meningitis have been changed as the result of streptomycin therapy, and it has been possible to study the electrolyte equilibrium of patients whose intake of food and water is adequate, and to observe changes during periods of remission and relapse.

The present study consists of serial determinations of plasma and red blood cell electrolytes in 16 children with tuberculous meningitis treated with streptomycin. In addition, analyses were made of samples of skeletal muscle obtained by biopsy in four patients at times when the concentrations of serum sodium and chloride were reduced. Samples of skeletal muscle were also obtained postmortem from patients who died of tuberculous meningitis. As controls, specimens of muscle were taken at operation from children without known infection, and obtained postmortem from children who died of a variety of diseases but without evidence of tuberculosis.

METHODS

Venous blood was drawn in syringes, moistened with a film of heparin solution, from patients in the postabsorptive state. A portion of the blood was immediately transferred to a centrifuge tube under a layer of mineral oil and the plasma separated by centrifugalization. The remainder was used for analysis of whole blood. Aliquots of plasma and whole blood were taken for water, chloride, sodium, and potassium determinations. Water was determined by drying a weighed aliquot of measured volume to constant weight in an oven at 105° C.; chloride by the method of Van Slyke and Sendroy (6). For determinations of sodium and potassium, aliquots of whole blood and plasma were measured into platinum dishes to which were added 3 drops of conc. HCl and then ashed at 450° C. in a muffle furnace. The ash was dissolved in a measured volume of distilled water, the insoluble residue was separated by centrifugalization, and the sodium and potassium content was determined by the use of a flame photometer (Perkin-Elmer, model 18). The blood cell volume was measured by centrifugalization in a Wintrobe tube for 30 minutes at 2,500 r.p.m. The concentration of solutes in red blood cells was calculated by the usual formula:

$$C = \frac{B - P(1 - V)}{V},$$

where C represents concentration in the red cell, B, concentration in whole blood, P, concentration in plasma and V, volume of cells in whole blood.

The specimens of skeletal muscle were dissected free of visible fat and connective tissue, then weighed and dried to constant weight in an oven at 105° C. The dried material was powdered and aliquots were taken for measurements of chloride, sodium, potassium, and nitrogen. Nitrogen was determined by a micro-Kjeldahl method; chloride, sodium, and potassium by methods similar to those used for plasma and whole blood. In a small group of patients, total body water was estimated by the antipyrine method (7), plasma volume by the Evans Blue technique (8), and the SCN space also determined (9). Simultaneous clearances of endogenous creatinine (10),

[1] Read by title before the American Society for Clinical Investigation, May, 1951.

TABLE I

Serum electrolytes of children showing marked reduction of concentrations of sodium and chloride during course of tuberculous meningitis

Patient	Serum							
	Na	Cl	CO_2	K	Ca	P	Urea N	Protein (gm./100 cc.)
	(mEq./liter)				(mg./100 cc.)			
P. C.	122	84.8	22.6	4.9	8.8	3.6	11.6	6.8
N. P.	119	81.6	24.3	—	9.1	4.2	15.6	7.3
V. B.	124	88.8	24.0	4.0	—	—	14.2	—
E. J.	124	89.6	22.4	5.3	—	—	17.1	—

sodium, chloride, and potassium were also measured in two patients.

TABLE II

Comparison of average values for serum electrolytes of patients with tuberculous meningitis during stages of clinical activity and remission

Group	Serum			
	Na	Cl	CO_2	K
	(mEq./liter)			
Tbc. meningitis active (15)*	127	92	21.6	4.6
Tbc. meningitis remission (8)	138	103	22.1	4.2
Controls (8)	140	105	—	4.4

* Number of patients in group in parentheses.

RESULTS

Representative data on children with tuberculous meningitis are shown in Table I. These are the results of determinations made several days after admission to the hospital at a time when the patients were receiving either a liquid diet, or solutions of sodium chloride parenterally. The patients at this stage showed no clinical evidence of dehydration although they were febrile and acutely sick. The analyses reveal a reduction of plasma sodium and chloride with relatively normal values for plasma bicarbonate, potassium, urea nitrogen, and total protein. The serum calcium is in the low normal range and the serum phosphorus is reduced below the normal values for children.

The results of determinations of Evans Blue space, SCN space, and antipyrine space as indices of plasma volume, "extracellular fluid" volume, and total body water, respectively, which were made in several patients with low concentrations of serum sodium, show no evidence of reduction of these compartments of body water. The Evans Blue space ranged from 4.3 to 6.8% of the body weight, the SCN space from 26% to 35% of body weight, and the values for antipyrine space in two children were 77% and 78% of the body weight. The volume of distribution of SCN cannot be used as a measure of actual extracellular fluid volume, but the high values obtained suggest that extracellular fluid volume in these patients is not reduced despite reduction of the concentration of extracellular sodium. The estimated values for total body water are also high, which may be due in part to the low body fat of these chronically ill children.

In Table II are given the average values for serum sodium, chloride, potassium, and bicarbonate of a group of patients with tuberculous meningitis during the clinically active stage in comparison with the findings in children during a period of clinical remission. The return of serum sodium and chloride to normal values during remission without change in serum potassium or bicarbonate

TABLE III

Clearances of endogenous creatinine, sodium, chloride, and potassium in children with tuberculous meningitis

Patient	Period	Urine output (cc./min.)	Serum			Clearances			
			Na	Cl	K	Creat.	Na	Cl	K
			(mEq./liter)			(cc./min./1.73 sq.m.)			
E. J.	1	1.9	126	88.8	3.0	123	0.42	0.69	21.8
	2	7.9				130	0.51	0.87	26.4
V. B.	1	2.8	120	76.8	3.4	133	1.85	4.10	51.3
	2	2.4				155	1.76	3.67	46.0

is evident. In addition, clearances of endogenous creatinine, sodium, chloride, and potassium were determined in two patients with tuberculous meningitis, at times when the concentration of serum sodium was low. The studies were made in the fasting state and the urine output was increased by oral administration of water. These data are summarized in Table III. The glomerular filtration rates, as measured by the endogenous creatinine clearance, are normal despite the marked reduction of serum sodium. The average percentage of filtered sodium reabsorbed was calculated to be 99.6% and 98.7% for E. J. and V. B., respectively. Although these values for tubular reabsorption of sodium suggest fairly complete reabsorption of sodium, it must be remembered that the normal human subject depleted of sodium shows almost complete tubular reabsorption of sodium with reduction of urinary sodium excretion to values less than those found in these patients. When the low concentrations of serum sodium of these patients are taken into consideration, the data are in accord with the studies of Rapoport, West, and Brodsky (11) who found excessive urinary losses of sodium in three children with tuberculous meningitis, and with the conclusions of Sims, Welt, Orloff, and Needham (5) that renal wastage of sodium and chloride may occur in pulmonary tuberculosis without evidences of adrenal insufficiency.

In Table IV are presented the concentrations of sodium and potassium in plasma water and in red cell water as determined in groups of healthy adults, control children, children with clinically active tuberculous meningitis, and children following a clinical remission from tuberculous meningitis. The control children ranged from four months to 16 years of age and were either convalescent from an illness or well children seen in the out patient department. The values for red cell potassium in these children agree with those reported in normal adults by Hald when these data are recalculated in terms of cell water (13). The concentrations of sodium in the red cells are quite variable and the average is lower than that found in adults (13). For this reason we made similar determinations in a group of healthy adults, and as shown in Table IV, the average concentration of red cell sodium in this group is higher than in the control children and of the same order of magnitude as in Hald's series (13). Overman, Etteldorf, Bass, and Horn (14) determined the sodium of packed red blood cell suspensions of infants and children from birth to two years of age and found the values to be the same or even slightly higher than those in adults studied by the same method. The method used by this group includes the sodium of an irreducible minimum of plasma incorporated in the red cell suspension. No explanation is apparent for the lower concentration of red cell sodium in children than in adults in our series. Since the patients with tuberculous meningitis fall into the same age group as the control

TABLE IV

Average concentrations of sodium and potassium in the water of plasma and of red blood cells of patients with tuberculous meningitis as compared with controls

Group	No.	Plasma water			Red cell water			$\frac{(Na + K)_c}{(Na + K)_p}$
		Na	K	Na + K	Na	K	Na + K	
		(*mEq./liter*)			(*mEq./liter*)			
Adult controls	8	152.6	5.0	157.9	20.7±3.3*	144±9.1	166.1	1.05±.064
					p<.01†			
Children controls	17	150.5	4.9	155.5	8.7±7.0	144.2±15.5	155.2	1.00±.084
					p<.01			p<.01
Tuberculous meningitis active	16	138.5	4.8	143.3	47.0±5.2	144.7±15.4	191.9	1.33±.16
Tuberculous meningitis remission	5	149.4	4.5	153.9	4.7	144.0	148.7	0.97

* Mean ± standard deviation.

† Statistical significance of difference between two means calculated by Fisher's method for small samples using table of T (12). Values of p of less than .05 are considered significant.

TABLE V

Concentrations of sodium and potassium in plasma and red cell water of children with non-tuberculous infections of central nervous system

Patient	Diagnosis	Plasma water			Red cell water			$\frac{(Na + K)_c}{(Na + K)_p}$
		Na	K	Na + K	Na	K	Na + K	
		(*mEq./liter*)			(*mEq./liter*)			
J. G.	Bulbar poliomyelitis	148	4.7	152.7	3.0	127	130	0.85
A. C.	Bulbar poliomyelitis	146	4.5	150.5	6.3	151	157	1.04
C. F.	Meningococcus meningitis	128	4.4	132.4	0	125	125	0.94

children and the determinations in the patients were made concurrently with the controls, the data in patients with tuberculous meningitis are compared with the values in the control children.

In the children with active tuberculous meningitis there is the expected reduction in the concentration of sodium in plasma water but in the red cell water there is an increase in the concentration of sodium without any change in the concentration of red cell potassium. The sum of the concentration of the univalent cations in red cell water is therefore higher than the normal value. The ratio of the sum of the concentrations of sodium and potassium in cell water to that in plasma water is given in the last column. This ratio which is close to unity in the controls is increased to an average value of 1.33 in the patients with tuberculous meningitis. During clinical remissions the plasma sodium rises to the normal value and the red cell sodium decreases so that the ratio of the concentration of univalent cations in cell water to that in plasma water is again in the normal range. The return of serum and red cell electrolytes to normal values during clinical remission does not necessarily indicate cure. Of the five patients studied during remission, two relapsed and the electrolyte changes characteristic of active tuberculous meningitis were again found.

Determinations of plasma and red cell sodium and potassium were also made in two children with bulbar poliomyelitis and one child with meningococcus meningitis, to see whether changes similar to those found in tuberculous meningitis might not occur in acute infections of the central nervous system due to other etiological agents. These data are shown in Table V. It is apparent that no increase in red cell sodium or total univalent cation is found in these patients. The findings in the child with meningococcus meningitis are of especial interest because the concentrations of sodium and of chloride in plasma are low, but the red blood cell sodium is not increased as in the patients with tuberculous meningitis.

Table VI summarizes the data obtained as the result of analysis of biopsy samples of skeletal muscle from patients with active tuberculous meningitis and from control children without known infection. All of the analyses are expressed in

TABLE VI

Electrolyte content of skeletal muscle obtained at biopsy from patients with tuberculous meningitis and from controls
All data expressed in terms of dried weight of muscle

Group	No. of pts.	Cl	Na	K	N	H_2O	Na/Cl	K/N
		(*mEq./100 gm.*)			(*gm./100 gm.*)			
Tuberculous meningitis	4	14.8	27.1 (23.2–30.6)*	21.7 (18.2–24.3)	11.9	305	1.84 (1.78–1.90)	1.81 (1.68–2.04)
Controls	6	13.8	18.6 (10.0–20.4)	34.1 (31.6–40.0)	11.9	349	1.37 (1.29–1.43)	2.89 (2.72–3.17)

* Range of values in parentheses.

TABLE VII

Electrolyte content of skeletal muscle obtained postmortem from children with tuberculous meningitis, and from children who died of other causes

All data expressed in terms of dried muscle

Group	No. of pts.	Cl	Na	K	N	H_2O	Na/Cl	K/N
		(*mEq./100 gm.*)			(*gm./100 gm.*)			
Controls (non-edematous)	5	11.8	15.3	37.0	13.9	365	1.28	2.71
Controls (edematous)	3	16.5	21.9	41.4	14.7	506	1.37	2.81
All controls	8						1.30±0.18	2.75±0.18
							$p<.01$	$p<.01$
Tuberculous meningitis	7	15.5	32.8	24.9	13.6	331	2.18±0.42	1.83±0.21

terms of dried muscle and no correction is made for fat content. In the children with tuberculous meningitis, the muscle sodium is higher and the muscle potassium considerably lower than in the controls. An elevated muscle sodium can be due to edema of muscle with an increased extracellular phase or to accumulation of sodium in the muscle fiber. The ratio of sodium to chloride can be used as a method of differentiating between these two causes of rise of muscle sodium. The increased ratio of sodium to chloride found in the muscle of patients with tuberculous meningitis indicates accumulation of sodium in the intracellular phase of the muscle. The ratio of potassium to muscle nitrogen can likewise be considered an index of change of concentration of cell potassium with respect to cell protein. The reduction of the ratio of potassium to nitrogen in the muscle of patients with tuberculous meningitis, shown in Table VI, thus indicates depletion of cell potassium. These muscle samples were obtained from patients at times when the concentrations of sodium in the serum were low, and those of potassium were in the normal range.

Additional studies of muscle were made on specimens obtained postmortem from patients who died of tuberculous meningitis and compared with analyses of muscle obtained from patients who died of other chronic diseases (Table VII). The possibility of postmortem change and the variable amounts of edema in some of these specimens made these analyses less satisfactory than those of the biopsy samples. However, the sodium to chloride and the potassium to nitrogen ratios of muscle obtained from children who died of a wide variety of diseases other than tuberculous meningitis are in the same range as the values obtained in biopsy specimens of the control group. Several of the control postmortem specimens were obtained from children who were severely cachectic as the result of central nervous system injury. The muscle analyses, however, show no abnormalities in contrast to the findings in the muscle of children who died of tuberculous meningitis. In the latter patients there is found the increase in the ratio of sodium to chloride and the decrease in the proportion of potassium to nitrogen which were obtained on analyses of skeletal muscle at biopsy in children with tuberculous meningitis.

DISCUSSION

The studies reported here reveal a characteristic pattern of disturbance of electrolyte equilibrium in patients with tuberculous meningitis. In extracellular fluid there is a reduction of the concentrations of sodium and chloride without consistent change in the concentration of potassium; in red blood cell water there is an increased concentration of sodium and no change in potassium so that the concentration of total univalent cations is increased; in muscle water there is an increase of sodium with a concomitant decrease of potassium. The average value for serum bicarbonate in these patients is slightly lower than the value of 24 mEq./liter obtained in normal children. Plasma pH measurements were not made and it is not possible to say whether this represents a mild metabolic acidosis or a respiratory alkalosis. The changes in extracellular electrolyte are similar to those seen at times in patients with pulmonary tuberculosis, usually those with advanced disease and marked temperature fluctuations (3, 4). No stud-

ies of intracellular electrolyte in pulmonary tuberculosis have been reported, but we have studied the red cell electrolytes in two patients with pulmonary tuberculosis without meningitis. In both of these patients there was found a reduction of serum sodium and an increase of red cell sodium with an elevation of total univalent cation in red cell water. To this extent the electrolyte disturbances observed in tuberculous meningitis and in pulmonary tuberculosis are similar although seen more consistently in the former group. Somewhat similar changes have been reported in other diseases. Overman found an increase of sodium in the red cells of monkeys (15) and of chicks (16) with experimental malaria, and suggestive but less definite changes in patients with induced malaria (17). Eliel, Pearson, and Rawson included a few analyses of red blood cell electrolytes in a study of electrolyte disturbances in the postoperative state (18). They found that patients subjected to extensive operative procedures showed a reduction of serum potassium and of chloride with normal serum sodium and an elevated serum bicarbonate. In the red blood cell there was evidence in a few cases of increased concentration of sodium which was associated with a possible decrease of potassium.

Loss of potassium from muscle cells with increase in muscle sodium can be produced experimentally in animals by a diet deficient in potassium (19) and may occur in disease states in which depletion of body potassium results from low intake of potassium, excessive loss of potassium from the body, or both (20–22). Potassium deficit may be produced by excessive adrenal function as in Cushing's disease (23), or by administration of steroids which increase the urinary excretion of potassium (19). In these states the serum potassium is reduced if no complicating state of dehydration is superimposed. Darrow and co-workers (19, 24) have shown that a metabolic alkalosis due to chloride deficit results in a loss of muscle potassium and an increase in muscle sodium, and, conversely, that experimentally produced deficiency of cellular potassium is associated with a decrease in serum chloride and a rise of serum bicarbonate with a resulting metabolic alkalosis. Hypochloremia and elevated serum bicarbonate are seen in patients with potassium deficiency particularly under conditions of ample or excessive sodium intake, and administration of potassium to such subjects is followed by a return of the serum electrolytes to normal. In our patients with tuberculous meningitis the concentration of serum bicarbonate was not increased and that of serum potassium was usually normal although low values were occasionally found. Following the administration of large amounts of sodium chloride to these children, a rise of the serum sodium and chloride values and, in some instances, an increased volume of extracellular fluid with visible edema did result, but the picture of hypochloremic alkalosis did not occur. Rapoport, West, and Brodsky (11) found that the values for serum bicarbonate and pH were high in two of three children with tuberculous meningitis. Their patients were in a terminal state and were receiving all fluids parenterally and did manifest the pattern of metabolic alkalosis with potassium deficit. The administration to several of our patients of potassium chloride in daily dosage of 3 mEq./kg. in addition to dietary intake of potassium did not result in any consistent restoration of the normal electrolyte pattern of either red blood cells or serum. This failure of potassium administration to correct the electrolyte pattern speaks against the possibility that these disturbances of the ionic equilibrium of extracellular and intracellular fluids are simply the result of loss of cell potassium which might result from increased adrenal cortical function in response to infection or other stimulus.

Peters and associates (25) have reported striking reduction of serum sodium and chloride in several patients with encephalitis and other lesions of the central nervous system. Studies of urinary excretion indicated that the depletion of extracellular electrolyte was due to loss of these ions in the urine. They suggested that injury to a nervous system center influencing water and electrolyte reabsorption by the renal tubule might explain the findings of reduced concentrations of serum sodium in this group of patients. The similarity between the findings in these patients and those in the children with tuberculous meningitis is suggestive, although Peters and co-workers pointed out that their patients showed some evidence of the characteristic physiological disturbances associated with reduction of extracellular sodium.

Interpretation of the changes in the concentrations of intracellular electrolytes found in the patients with tuberculous meningitis is hampered by

the incomplete knowledge of the mechanisms controlling ionic equilibrium between cells and extracellular fluid in the normal state. In recent years Conway (*26*), Ussing (*27*), and Sheppard (*28*) among others have reviewed the evidence concerning the physiological processes involved in the maintenance of potassium gradients between cells and surrounding media. Conway has supported the hypothesis that the cell membrane is permeable to the hydrated potassium ion but not to the larger hydrated sodium ion. The concentration gradient of potassium can then be explained in terms of a double Donnan equilibrium resulting from the presence of non-penetrating anions within the cell and a non-penetrating cation outside the cell. On this basis little energy expenditure is required to maintain a concentration gradient between cell and surrounding medium.

On the other hand the evidence summarized by Ussing (*27*) indicates that sodium and potassium can both diffuse through the cell membrane. This has led to the hypothesis that exclusion of sodium from cells normally is due to an active secretory process which extrudes sodium from the cell at a rate which balances the inward diffusion of sodium. The end result is similar to that which would obtain if the cell surface were impermeable to sodium and permeable to potassium, but the extrusion of sodium from the cell against a concentration gradient requires the expenditure of energy.

The studies of human red cells under *in vitro* conditions which have been reviewed by Sheppard (*28*) have revealed reversible increase of cell sodium and decrease of cell potassium depending upon the environmental conditions. The results of such experiments are compatible with the postulation that inhibition of cellular metabolic processes, necessary for active removal of sodium from the cell, results in accumulation of sodium with loss of potassium, and that restoration of cell metabolism permits removal of the accumulated sodium from the cell with uptake of potassium.

In the skeletal muscle of the patients with active tuberculous meningitis increase of sodium is associated with loss of potassium. In the red blood cells accumulation of sodium is found without loss of cell potassium, so that the total concentration of univalent cations in cell water is increased although the concentration of cations in plasma is decreased. Since osmotic equilibrium between red cells and plasma is assumed to be present it is necessary to presuppose either a compensating reduction of osmolar concentration of other solutes in the red cell, or else that a fraction of the cell cation is unionized and osmotically inactive. Reduction of the osmolar concentration of anions could occur if an increased proportion of the cell base was neutralized by polyvalent anions of high molecular weight with a decrease in the concentration of univalent anions. The chief univalent anions of the red blood cell are bicarbonate and chloride. Cell bicarbonate was not measured, but determinations of cell chloride showed that the average concentration of this ion was essentially the same in the high sodium as in the low sodium red cells, approximately 75 mEq./liter of red cell water. It is unlikely then that increased cation concentration can be offset by reduction of the osmolar concentration of anions. Little is known of the existence of unionized complexes of alkali metals in biological systems. Steinbach (*29*) has concluded that frog muscle homogenates bind a small fraction of sodium in an undissociated form, but there is no direct evidence for the existence of unionized complexes of sodium or potassium in the red cell.

The reduction of the concentration of extracellular sodium and the consequent reduction of the osmotic pressure of the extracellular fluid may be an adaptation to reduction of osmotic pressure within the cell due to changes in the state of intracellular electrolyte in tissue cells as well as in the red blood cell. Primary changes in cell osmolarity as the basis for changes in concentrations of electrolytes in extracellular fluids have been suggested by Welt, Orloff, Kydd, and Ottman (*30*), and Sims, Welt, Orloff, and Needham (*5*). It is conceivable that in the renal tubule cells a disturbance of sodium equilibrium analogous to that found in muscle and red blood cell may be associated with diminished tubular reabsorption of sodium and of chloride. The possibility that tuberculous infection and other disease processes may cause inhibition of cellular processes necessary for maintenance of electrolyte equilibrium between cells and extracellular fluid, and thus produce the changes in concentrations of extracellular and intracellular

electrolytes described above is of course speculative. Increased knowledge of the mechanisms of ionic equilibrium between cells and extracellular fluid in the normal state will help in the interpretation of the findings in disease states.

Despite the abnormal concentrations of both intracellular and extracellular electrolytes in the body fluids of patients with tuberculous meningitis, efforts at correction of these disturbances have not been rewarded by any apparent therapeutic effect. As pointed out above, despite the reduction of extracellular sodium there is no evidence of reduction of extracellular fluid volume. The measurements of SCN space cannot be regarded as a true index of extracellular fluid volume and the high values for total body water (antipyrine space) do not preclude shifts of water from the extracellular to the intracellular compartments. However, the normal values for plasma volume, as indicated by Evans Blue space, and glomerular filtration rate, as measured by creatinine clearance, in the few patients studied speaks against any considerable reduction of extracellular fluid volume. The absence of the usual clinical evidences of dehydration is in accord with these laboratory findings. When large amounts of sodium chloride were added to the ordinary intake of these children, increased concentrations of sodium and of chloride resulted with development of edema but without evidence of symptomatic improvement. During the active phase of the disease, the symptoms due to the tuberculous meningitis are usually so marked that it is not possible to say whether there are any clinical manifestations associated with the depletion of intracellular potassium. No significant effects were noted in three patients to whom extra potassium chloride was given in a dosage of 3 mEq./kg./day.

SUMMARY

Determinations of the concentrations of electrolytes in the plasma, red blood cells and skeletal muscle of children with tuberculous meningitis have revealed abnormalities of the electrolyte pattern of both extracellular and intracellular fluids. During the stage of active infection the following changes are found:

In the plasma the concentrations of sodium and chloride are reduced without any consistent change in the concentration of potassium. These losses of extracellular electrolytes are not accompanied by any evidences of reduction of extracellular fluid volume.

In red blood cells the concentration of sodium is increased and that of potassium unchanged. The concentration of total univalent cation in red cell water, and the ratio of the sum of the concentrations of sodium and potassium in red cell water to that in plasma water are therefore increased.

In skeletal muscle the sodium content is raised and the potassium content reduced. The increase of the ratio of sodium to chloride in muscle suggests accumulation of sodium in the muscle fiber while the reduction of the ratio of potassium to nitrogen indicates depletion of cell potassium.

During the stage of clinical remission the concentrations of sodium and of chloride in plasma, and of sodium in red blood cells return to the normal range.

In severe tuberculous infections there is a disturbance of the mechanisms governing ionic equilibrium between cells and extracellular fluid. Renal loss of sodium and of chloride with consequent reduction of the concentrations of these ions in extracellular fluid may be one aspect of this more general abnormality.

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VALIDITY OF THE ENDOGENOUS CREATININE CLEARANCE AS A MEASURE OF GLOMERULAR FILTRATION RATE IN THE DISEASED HUMAN KIDNEY [1, 2]

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INTRODUCTION

Twenty-five years ago Rehberg proposed the exogenous creatinine clearance for the measurement of glomerular filtration rate (1). In the *dog* and *rabbit,* this clearance provides a simple and accurate method of determining the filtration rate, coinciding with the inulin and ferrocyanide clearances (2, 3). Subsequently, Shannon (4) and Miller and Winkler (5) found *in man* that the exogenous creatinine clearance was considerably higher than the glomerular filtration rate.[6] Despite the fact that the compound he chose did not provide an exact measure of the glomerular clearance in man, Rehberg's pioneering development opened a whole new area of research in renal dynamics. Unfortunately, the unreliability of *exogenous* creatinine and ferrocyanide (7) clearances in man left only inulin for the measurement of filtration rate in the human kidney.

The determination of inulin clearance has several serious disadvantages. It was inevitable, therefore, that the search for simpler methods of measuring glomerular filtration rate in man would continue. It occurred to one of us some years ago that the *endogenous* creatinine clearance might prove to be a true measure of the filtration rate.

Miller and Dubos developed a specific enzymatic method for the estimation of creatinine (8, 9), and this method was employed by Miller and Winkler to measure the clearance of *specific endogenous creatinine* in normal and pathological states (5). They demonstrated that in *normal* individuals the specific endogenous creatinine clearance lay below the level of Rehberg's exogenous clearance. In some instances, it equalled the inulin clearance. In addition, these authors found that the specific endogenous creatinine clearance was consistently higher than the inulin clearance in patients with diminished renal function, as in chronic glomerulonephritis. Further studies by Taggart, Alving and Miller (10, 11), employing a more sensitive colorimeter, showed that the specific endogenous creatinine clearance in both normal and hypertensive subjects was almost always somewhat higher than the inulin clearance.

Other workers, using non-specific methods for analysis, have claimed that the endogenous creatinine chromogen clearance may be employed as a measure of glomerular filtration rate in man. These results have been summarized by Smith (2).

Brod and Sirota reported a high degree of correspondence between endogenous-creatinine-chromogen and inulin clearances in normal subjects and patients with renal disease (12). Because of the simplicity of the endogenous creatinine clearance, there has been a tendency for many investigators to substitute this clearance for the inulin clearance. In view of the discrepancy between the results of Miller and Winkler (5) and Taggart, Alving and Miller (10, 11), and those obtained by Brod and Sirota on the validity of the creatinine clearance, it seemed important to extend these studies. We have therefore determined both the specific creatinine clearance as described by Miller and Winkler and the non-specific creatinine chromogen

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[2] A preliminary report of this work appeared in the Proceedings of the Forty-Third Annual Meeting of the American Society for Clinical Investigation, J. Clin. Invest., 1951, **30**, 661.

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[6] The inulin clearance value is regarded as equivalent to the glomerular filtration rate in man (2, 6).

clearance, used by Brod and Sirota. For the most part, our subjects were patients who presented evidence of considerable renal derangement, either organic as a result of chronic inflammatory disease, or functional, from cardiac failure. We have purposely selected patients with considerable renal pathology since we feel that general acceptability of any glomerular clearance measurement depends on its equivalence with the clearance of inulin in this type of patient.

METHODS

The clearances of specific endogenous creatinine, total creatinine chromogen and inulin were determined on nine patients. Prior to the collection of samples, these patients had received 5 grams of allantoin by mouth.[7] We have no reason to suspect that allantoin modifies either the inulin or creatinine clearance.

The inulin clearance was obtained by the method described by Goldring and Chasis (14) which employs a priming dose of inulin[8] and a continuous intravenous infusion to ensure a constant blood level.

Blood samples were centrifuged as soon as they had clotted, and the serum was withdrawn. Sera and urines were stored in the frozen state if they were not analyzed immediately. All analyses were performed in duplicate.

Inulin

Inulin was determined by the Rolf, Surtshin and White (15) modification of the Alving, Rubin and Miller diphenyl amine method (16). Sera were yeasted according to the method of Alving, Rubin and Miller only in experiments where inulin had been administered in a 2 per cent glucose solution to the patients with congestive heart failure.

Blood sera were deproteinized by acid cadmium sulfate (17), as were urines which contained protein. Aliquots of blood filtrates and diluted urines, containing approximately 20 micrograms of inulin in 2 cc., were analyzed in duplicate along with 20 microgram standards, freshly prepared from a stock solution containing 1 mg. per cc.

A blood sample drawn prior to the administration of inulin served to correct for glucose in subsequent blood samples. A control urine sample was also collected just before the priming dose of inulin. This gave a measure of non-inulin reducing substance in urine, and was used to correct the values obtained on urines collected during the clearance periods. Generally, the control urines contributed very little color. However, in urine from uremic patients, the correction is considerably greater, as has been observed by other investigators (12).

[7] Allantoin was administered to obtain the allantoin clearances reported in the accompanying paper (13).

[8] We are indebted to the U. S. Standard Products Company, Woodworth, Wisconsin, for a generous supply of inulin.

Creatinine

Both total creatinine chromogen (*apparent* creatinine) and non-creatinine chromogen (the chromogen remaining after treatment with the Miller-Dubos enzyme) were determined by the Bonsnes and Taussky (18) modification of the Jaffe reaction. Serum proteins were precipitated by the addition of 8 cc. of water, 0.5 cc. 10 per cent sodium tungstate and 0.5 cc. ⅔ N H_2SO_4 to 1 cc. of serum in the usual manner. Urine samples which contained protein were also precipitated with tungstate-sulfuric acid (5 cc. of urine plus 1 cc. of 10 per cent sodium tungstate and 1 cc. of ⅔ N H_2SO_4). To 6 cc. of serum filtrate or appropriately diluted urine, 2 cc. of 0.04 M picric acid were added. The tubes were placed in a 28° C. water bath, and 2 cc. of 0.75 N NaOH were added at carefully timed intervals. The color formed was read in an Evelyn colorimeter with filter 520 after exactly 15 minutes, and the total creatinine chromogen was determined by reference to a standard curve.

For the determination of specific creatinine, 6 cc. of blood filtrate or diluted urine were incubated with 1 cc. of water, 0.5 cc. molar phosphate buffer, pH 7.0, and 0.5 cc. of bacterial suspension (19) in an unstoppered Erlenmeyer flask for 1 hour at 37° C. The contents of the flask were centrifuged at high speed for 15 minutes, and the residual chromogen (non-creatinine chromogen) was determined on 6 cc. of the supernatant fluid, exactly as described above. The difference between total creatinine chromogen and non-creatinine chromogen represents specific creatinine.

RESULTS

Table I summarizes the clearances obtained in nine patients. The ratio of *total creatinine chromogen* clearance to inulin clearance permits comparison with the results of Brod and Sirota (12) who employed this measure of "creatinine." Also, the ratios of *specific creatinine* clearance to inulin clearance are reported.

Total creatinine chromogen clearance

The ratios of total creatinine chromogen clearance to inulin clearance fell within 10 per cent of unity in only five of 28 clearance periods. The remainder ranged from 0.56 to 1.64. Two of our three patients with rheumatic heart disease and cardiac failure exhibited the very low total creatinine chromogen to inulin clearance ratios reported by Baldwin, Sirota and Villarreal (20) in cardiac decompensation. In patient B. M., the ratios of 0.58, 0.56, and 0.63 are remarkably low.

Specific creatinine clearance

These clearances show the same trend as the non-specific ones. In all but one case, they have

TABLE I

Comparison of inulin and endogenous creatinine clearances

Patient	Sex	Age	Diagnosis	Period	Urine flow (cc./min.)	Inulin		Creatinine chromogen		Specific creatinine		Clearance ratios	
						Serum conc'n (mg./100 cc.)	Clearance* (cc./min.)	Serum conc'n (mg./100 cc.)	Clearance (cc./min.)	Serum conc'n (mg./100 cc.)	Clearance (cc./min.)	Creatinine chromogen/inulin	Specific creatinine/inulin
S. A. S.	M	49	Hyperparathyroidism with nephrocalcinosis	1	3.3	12.4	11.9	4.02	19.2	3.81	20.3	1.61	1.71
				2	3.1	14.0	10.5	4.02	17.2	3.80	18.2	1.64	1.73
				3	3.5	14.7	11.6	4.00	17.5	3.78	18.5	1.51	1.59
M. W.	F	23	Lupus erythematosus with severe hypertension	1	3.2	19.6	22.3	3.24	28.2	2.78	30.6	1.27	1.37
				2	2.9	21.4	21.0	3.24	26.1	2.82	28.0	1.24	1.33
				3	3.4	23.8	22.2	3.25	27.7	2.78	31.3	1.25	1.41
R. P.	M	35	Diabetes with intercapillary glomerular sclerosis	1	5.2	23.5	53.0	1.57	65.9	1.20	74.8	1.24	1.41
				2	6.5	23.6	54.4	1.54	66.5	1.13	76.9	1.22	1.41
				3	7.7	23.7	58.2	1.56	65.1	1.14	74.6	1.12	1.28
				4	8.1	23.0	58.6	1.48	64.7	1.13	77.6	1.10	1.33
A. M.	M	52	Chronic glomerular nephritis with nephrosis	1	1.2	39.5	56.6	1.38	81.5	1.19	91.0	1.43	1.60
				2	1.5	43.6	56.2	1.37	77.5	1.18	86.8	1.38	1.54
				3	1.1	48.4	55.1	1.31	83.6	1.12	94.9	1.52	1.72
A. J.	F	26	Anterior and postpituitary deficiency	1	6.2	49.1	66.6	0.88	88.1	0.66	115.5	1.32	1.74
				2	5.7	52.6	58.1	0.91	70.1	0.66	94.6	1.21	1.63
				3	6.2	57.2	64.9	0.92	73.5	0.65	103.8	1.13	1.60
A. L.	M	45	Rheumatic heart disease	1	0.6	30.0	78.6	0.96	50.6	0.70	65.0	0.64	0.83
				2	0.6	28.6	80.7	0.91	55.1	0.65	72.3	0.68	0.90
				3	0.6	29.2	82.5	0.88	55.0	0.62	72.5	0.67	0.88
J. R.	M	21	Vitamin D-resistant rickets	1	9.0	51.4	101.5	0.99	105.0	0.99	105.0	1.03	1.03
				2	8.1	51.6	88.5	0.99	94.6	0.94	94.6	1.07	1.07
				3	8.5	51.8	86.4	0.97	92.9	0.97	92.9	1.07	1.07
F. B.	F	39	Rheumatic heart disease	1	8.1	30.4	103.8	0.83	90.4	0.51	134.2	0.87	1.29
				2	8.4	30.1	88.0	0.84	77.1	0.52	116.6	0.88	1.33
				3	7.5	29.7	86.1	0.89	78.4	0.57	112.0	0.91	1.30
B. M.	F	31	Rheumatic heart disease	1	0.7	22.6	95.0	0.72	55.5	0.46	77.5	0.58	0.82
				2	0.8	20.4	109.5	0.73	61.1	0.47	91.1	0.56	0.83
				3	0.9	21.4	114.0	0.69	72.1	0.43	108.0	0.63	0.95

* All clearances are corrected for body surface area of 1.73 sq. meters.

higher absolute values because the concentration of specific creatinine in serum is lower than that of total creatinine chromogen. The urinary creatinine values obtained by the two methods are essentially the same. The ratios of specific creatinine clearance to inulin clearance also deviate considerably from the required value of unity. The lowest specific creatinine/inulin ratio was 0.82, and the highest, 1.74.

DISCUSSION

The clearance values observed in these patients substantiate the conclusion reached by Miller and Winkler in 1938 that the endogenous creatinine clearance in subjects with depressed renal function is usually higher than the inulin clearance (5). In patient S. A. S., who showed the greatest reduction in function, the specific creatinine/inulin ratios averaged 1.68 and the total creatinine chromogen/inulin ratios, 1.59. Similar results were obtained in patient A. M. whose kidney function was considerably better, but was still only 50 per cent of normal because of glomerular nephritis in the nephrotic stage. This patient was found to have specific creatinine/inulin ratios averaging 1.62, and the corresponding creatinine chromogen/inulin value of 1.45.

It is difficult to reconcile the data obtained in our present study or by Miller and Winkler (5) with the result reported by Brod and Sirota (12). These authors studied 13 adult patients with renal disease and found satisfactory correspondence be-

tween the creatinine chromogen and inulin clearances except in one patient, whose chromogen/inulin clearance ratio averaged 1.61.

Brod and Sirota themselves have emphasized that in the infant, the endogenous creatinine chromogen clearance is not valid (12). Baldwin, Sirota, and Villarreal (20) have also pointed out that the endogenous creatinine chromogen clearance is substantially lower than the inulin clearance in patients with congestive heart failure and edema. Also, Brod (21) described a patient suffering from malignant hypertension associated with chronic pyelonephritis who showed some endogenous creatinine clearance values that were markedly lower than inulin. Brod and Kořátko (22) found in some patients with renal disease that the clearance of endogenous creatinine lay well above the inulin clearance. In these subjects, caronamide tended to bring the endogenous creatinine/inulin clearance ratio down to unity. The ratios mentioned by Brod and Kořátko are fully as high as those reported by us in the present communication and, previously, by Miller and Winkler (5). Also, the demonstration that caronamide reduces the endogenous creatinine clearance provides additional evidence that tubular excretion of endogenous creatinine occurred in these patients. Similar observations in chronic renal insufficiency have been made by Hare and associates (23).

It is clear that both Brod and Sirota question the validity of their endogenous creatinine chromogen clearance as a measure of the filtration rate in (a) normal infants, (b) patients with congestive heart failure, and (c) some patients with renal disease. We feel that such numerous exceptions would make the endogenous creatinine chromogen clearance unreliable in any studies which demand a relatively high degree of accuracy. If one wishes only a practical test of renal function, it is questionable whether anything is gained by using the non-specific, endogenous creatinine chromogen clearance in place of the urea clearance.

Our results hold no greater hope for the use of the *specific endogenous creatinine clearance* as a precise measure of the filtration rate in man. This is unfortunate because relatively simple adsorption methods for the specific estimation of serum creatinine have been described (24–26).

It is conceivable that unknown factors in the clinical, therapeutic or dietary state of the subjects may influence the creatinine clearance. The deviations of the endogenous creatinine clearance from the glomerular filtration rate show so much variability that it would not be surprising to find that this clearance is affected by factors not ordinarily considered in clearance measurements.

Three of the patients included in our study had impaired renal function caused by congestive heart failure. In two of them, the non-specific creatinine clearance fell considerably below the inulin level. The specific creatinine clearances were also unexpectedly low. It seems likely, therefore, that the low endogenous creatinine chromogen clearances found in cardiac failure cannot be attributed to the presence of an unusually large quantity of non-creatinine chromogen in serum. The two patients who showed the low creatinine/inulin clearance ratios had very low urine flows (see Table I). Our data are too limited to permit any conclusions about the importance of this factor.

CONCLUSIONS

In patients with organic and functional disease of the kidney, the endogenous creatinine clearance is not a satisfactory measure of the glomerular filtration rate. This discrepancy between the creatinine and inulin clearances occurs with both the creatinine-chromogen clearance advocated by Brod and Sirota, and the specific creatinine clearance, determined by the enzymatic method of Miller and Dubos.

It is concluded that in patients with renal disease or in cardiac failure, endogenous creatinine clearance should not be employed as a precise measure of the glomerular filtration rate.

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A COMPARISON OF THE ALLANTOIN AND INULIN CLEARANCES FOR THE MEASUREMENT OF FILTRATION RATE IN THE DISEASED HUMAN KIDNEY [1, 2]

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A substance capable of measuring filtration rate with the precision of inulin, but without its drawbacks, would be extremely helpful in measurements on the human kidney. A suitable compound would be one which is already present in the body or easily administered by mouth. Allantoin appeared to answer these requirements. Its renal clearance had been measured in rats and dogs by Friedman and Byers (1), and it seemed to provide a precise measure of filtration rate. When administered orally it is non-toxic and not too unpalatable. It is relatively inexpensive. Furthermore, the allantoin reaches an adequate concentration in the blood stream after approximately two hours, and this level remains fairly constant for a period of three hours (2).

Friedman, Byers and Abrahm therefore studied the clearance of allantoin in a group of normal human subjects and in patients suffering from cardiorenal diseases (3). They concluded from their experiments that the allantoin clearance offers a precise measure of filtration rate in patients with both normal and diseased kidneys.

It seemed to us important to repeat and extend these observations on the allantoin clearance, especially in the diseased kidney. We have assumed that the inulin clearance may be taken as the "referee" value, and have compared inulin and allantoin clearances in nine patients with various types of renal lesions.

METHODS

The inulin clearance was obtained by the procedure described in the preceding paper (4). Allantoin clearances were performed as described by Friedman, Byers and Abrahm (3), and allantoin was determined according to their procedure. This is essentially the method of Christman, Foster and Esterer (5), with the incorporation of a modification proposed by Young and Conway (6). In our initial studies with this method, we obtained excellent recoveries of allantoin added to urine, but discouragingly erratic results with serum. This appeared to be caused by the use of full-strength Folin-Wu sodium tungstate and sulfuric acid reagents in the preparation of protein-free filtrates. Perfectly satisfactory results were obtained with half-strength reagents, employing 8 cc. of water, 0.5 cc. 10 per cent sodium tungstate and 0.5 cc. $\frac{2}{3}$ N H_2SO_4 for each cc. of serum. We have found the method accurate and reproducible, but exceedingly tedious and demanding when compared with the simplicity of creatinine or inulin determinations.

Blood samples were yeasted in all experiments, and urines were yeasted in one experiment on a diabetic patient.

To preclude the possibility of oxidation of uric acid to allantoin, blood sera and urines were kept in the frozen state until ready for analysis. They were analyzed immediately after the preparation of protein-free filtrates.

As indicated by Friedman and co-workers, uric acid yields about one-fifth as much color as allantoin. Allantoin values were therefore corrected for uric acid, which was determined by the method of Archibald (7).

RESULTS AND DISCUSSION

The inulin and allantoin clearances, and the allantoin/inulin ratios are presented in Table I. Simultaneous clearances of inulin and allantoin were obtained in 28 periods. In 11 comparisons the clearance ratios fell within 10 per cent of unity. In the others the deviations were greater. Seventeen clearance ratios of allantoin to inulin fell below

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[2] A preliminary report of this work appeared in the Proceedings of the Forty-Third Annual Meeting of the American Society for Clinical Investigation, J. Clin. Invest., 1951, **30**, 661.

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0.9; the lowest was 0.64. Eight clearance ratios lay between 0.7 and 0.8, and eight between 0.8 and 0.9.

The deviations of clearance ratios were not related to renal function. Patient S. A. S. who had the poorest excretory function showed allantoin/inulin ratios of 0.84, 0.75 and 0.77, and patient B. M. who had the highest inulin clearance in the group gave clearance ratios of 0.73, 0.75, and 0.64.

Low allantoin/inulin ratios were observed in patients with advanced organic disease of the kidney (S. A. S. and M. W.), as well as in patients with the reversible renal impairment of cardiac decompensation (A. L. and B. M.).

TABLE I

*Comparison of inulin and allantoin clearances**

Patient	Allantoin		Clearance ratio allantoin/inulin
	Serum conc'n (*mg./100 cc.*)	Clearance† (*cc./min.*)	
S. A. S.	1.79	10.0	0.84
	1.85	7.9	0.75
	1.87	8.9	0.77
M. W.	5.7	19.2	0.86
	5.8	17.3	0.82
	6.2	18.0	0.81
R. P.	6.8	57.1	1.08
	7.0	56.9	1.04
	6.9	54.1	0.93
	6.7	56.6	0.97
A. M.	5.4	57.1	1.01
	5.4	56.0	1.00
	5.5	59.8	1.08
A. J.	4.7	69.9	1.05
	4.8	57.1	0.98
	4.8	63.0	0.97
A. L.	4.6	62.0	0.79
	4.5	65.9	0.82
	4.5	70.0	0.85
J. R.	5.1	79.0	0.78
	5.4	67.0	0.76
	5.5	66.5	0.77
F. B.	6.4	90.0	0.87
	6.1	77.3	0.88
	5.7	81.1	0.94
B. M.	2.6	68.8	0.73
	2.8	82.1	0.75
	3.1	73.0	0.64

* See accompanying paper (4) for further details regarding these patients in whom the allantoin clearances were determined *simultaneously* with the inulin and creatinine clearances shown on p. 311.

† All clearances are corrected for body surface area of 1.73 sq. meters.

It is interesting that when good agreement was obtained between the allantoin and inulin clearances all comparisons in the individual experiments were close to unity. For example, patient R. P. had ratios of 1.08, 1.04, 0.93, and 0.97; patient A. M., 1.01, 1.00, and 1.08; and patient A. J., ratios of 1.05, 0.98, and 0.97. Unfortunately, we were not able to repeat the clearance experiments on any of the patients in the group to ascertain the degree of reproducibility of the clearance ratios at different times.

If allantoin behaved like inulin and were excreted entirely by filtration, the allantoin/inulin ratios should all approach unity. We have found this to be true in only three of nine experiments. The deviations of the allantoin clearance are all in the same direction, lower than inulin. This suggests that there is tubular reabsorption of allantoin in some individuals. Such a finding is perhaps not surprising for a molecule with such a low molecular weight compared with inulin.

Perhaps the study of a large number of patients with various types of renal lesions would establish certain patterns with respect to the reabsorption of allantoin. It is interesting that the three patients with cardiac failure showed consistently lower allantoin clearances than inulin. On the other hand, two of our patients with more severe renal disease had allantoin clearances that were identical with inulin. All but one of the inulin clearance values which Friedman and his collaborators reported for patients with cardio-renal disease exceeded 80 cc. per minute. It is possible that with subjects showing more advanced pathology, these investigators might also have observed larger deviations.

Because of the tediousness and exacting nature of the analytical method for allantoin, there would seem little justification for its use in clearance studies unless it were a reliable glomerular clearance which could be employed under all conditions. In human disease, the allantoin clearance appears to us to have decidedly limited applicability.

SUMMARY

In the human kidney which has been damaged by nephritis and other pathological conditions, the allantoin clearance does not consistently measure the filtration rate. The results suggest that allantoin undergoes partial tubular reabsorption in some, but not all, diseased kidneys.

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SOME ASPECTS OF THE HUMAN PHARMACOLOGY OF TETRAETHYLTHIURAMDISULPHIDE (ANTABUS) [1]–ALCOHOL REACTIONS [2]

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INTRODUCTION

In 1948, Hald, Jacobsen, Larsen, Asmussen, and Jørgensen working in Denmark published a series of five papers on the effect of tetraethylthiuramdisulphide alone and in combination with ethanol on man and animals (1–5). They reviewed and added to Hanzlik's and Irvine's (6) previous studies on the pharmacology and toxicology of Antabus. In addition these authors described the syndrome occurring during an alcohol-Antabus reaction, emphasizing in particular the cardiovascular and respiratory changes observed. These workers reported marked peripheral vasodilatation (evidenced by increase in skin temperature and flushing), a slightly increased cardiac output, an increase in oxygen consumption, increased ventilation, decreased alveolar carbon dioxide, increased respiratory dead space and increased pulse rate.

These authors next offered considerable evidence to support the theory that the reaction was due to the accumulation of acetaldehyde (assumed to be the first breakdown product of ethanol) in the body. They also postulated that the accumulation was due to increased production of acetaldehyde. Later Hald and Larsen (7) refuted the idea of increased production of acetaldehyde and stated that it was a decrease in the rate of metabolism of this agent that caused its accumulation in Antabus treated subjects.

Later Kjeldgaard (8) showed that Antabus inhibited aldehyde oxidase in concentrations as small as 0.1 microgram per ml. (*in vitro* purified enzyme experiment) thereby preventing the oxidation of aldehydes to acids. This inhibition offered a possible explanation for aldehyde accumulation in the blood of intact animals.

Much of this work was confirmed by Newman (9) in his experiments on dogs. He found that Antabus decreased the ability of the animals to oxidize acetaldehyde and found, as did the Danish workers, that intravenous infusion of acetaldehyde produced a syndrome similar to that occurring with Antabus-alcohol reactions.

Since the use of Antabus as a therapeutic agent for alcoholism has received considerable attention in recent years, this work was undertaken for the purpose of further studying the human pharmacology of Antabus-alcohol reactions.

PROCEDURE

A total of 51 patients was studied of which 41 were out-patients and 10 were hospitalized. These patients were the first 51 in a group of over 100 patients being treated for alcoholism at The Langley Porter Clinic, San Francisco (10). No patient who had any potentially serious organic defect was included in The Langley Porter Clinic study.

Before Antabus therapy was initiated, the status of each patient was evaluated by means of a physical and neurological examination, liver function test, chest x-ray, blood count, urinalysis, and electrocardiogram. In addition, psychiatric and psychologic studies were carried out. All laboratory studies were found to be within normal limits.

Out-patients were started on Antabus five days before their initial test drink and received 1 gram on the first and second days, 0.75 gram on the third and fourth days, and 0.5 gram on the fifth day (at least two hours before the initial test drink of alcohol was given). One-half gram was given as a daily maintenance dose thereafter except in a few cases when it was found necessary to increase or decrease the dosage. The dosage of alcohol used was 0.5 ml. per kg. of a 90 proof beverage. This dosage represents, in an average-sized man, the amount found in one jigger of bar whiskey.

[1] Antabus in the study was furnished through the courtesy of Ayerst, McKenna, and Harrison.

[2] Supported in part by a grant from the Committee on Research of the Medical Center, University of California at San Francisco.

[3] U. S. Public Health Fellow in Pharmacology.

Each patient had one member of the examining staff remain in attendance with him for at least an hour after the test drink was given to record the data listed below. In addition, one of the authors (E. M.) observed each patient during the entire test period to see that the signs and symptoms in the various patients were rated on an equal basis. An average of four patients was tested a week.

Pulse, respiration, and blood pressure were determined before the test drink was given and every five minutes for a period of an hour after. Blood samples for alcohol and acetaldehyde determinations were drawn in heparinized syringes before and at 10, 20, 30, 45, and 60 minutes after the drink. In subjects showing severe reactions blood samples were also drawn at 80 minutes. Airtight caps were placed on the ends of the syringes which were then immersed in an ice bath. As soon as the test period was over the samples were taken to the laboratory and the alcohol and acetaldehyde determinations made.

Blood alcohols were determined by a modification of the method described by Winnick (11) and blood acetaldehyde by a method developed in this laboratory (12).

An evaluation of the following signs and symptoms was made:

1. Blushing
2. Feeling of heat
3. Itching
4. Conjunctival injection
5. Throbbing (head)
6. Headache
7. Dyspnea
8. Palpitation
9. Dizziness
10. Nausea
11. Vomiting
12. Sweating
13. Cold shivers
14. Abnormal odor (breath)
15. General indisposition
16. Sleepiness
17. Fear
18. Convulsions

These signs and symptoms were listed on a check sheet followed by three spaces. In the first space was noted the time of onset, in the second the time of greatest intensity, and in the third space the observers' rating of the intensity; a mild reaction was rated as 1, a moderate reaction as 2, and a severe reaction as 3.

One week later most patients returned and received the same treatment with the exception that the dose of the alcoholic beverage was one-half of the previous dose.

The hospitalized patients used as a control group for this study were given the same dose of alcohol and the above data recorded prior to as well as after Antabus therapy.

RESULTS

Alcohol determinations

The normal mean alcohol blood level (before the ingestion of alcohol) of the 10 hospitalized patients before treatment with Antabus was 4.9 ± 3.3 mg. per cent.[4] The normal mean level for the 51 Antabus treated patients was 6.0 ± 3.0 mg. per cent. These data subjected to the student "t" test were found not to be significantly different ($p = 0.45$). Therefore we conclude that Antabus does not affect the normal alcohol "blank" value. Neither does Antabus affect the rate of absorption or rate of metabolism of alcohol and this is indicated by the blood levels determined at 10, 20, 30, 45, and 60 minute intervals after small doses of alcohol (see Table I and Figure 1). Curve A, Figure 1 is the "mean curve" for the untreated patients. Curve B is the "mean curve" for the treated patients receiving 0.5 ml. of alcohol per kg. body weight. Curve C is the curve for the treated pa-

[4] This figure includes all volatile oxidizable substances as determined under our experimental conditions and is not intended to represent only ethanol.

TABLE I

Mean increase in alcohol levels for treated and untreated patients after test drink

Time in Minutes	Untreated Patients 0.5 ml./kg.			Treated Patients 0.5 ml./kg.			Treated Patients 0.25 ml./kg.		
	Mean Increase	Stnd. Dev.	Range	Mean Increase	Stnd. Dev.	Range	Mean Increase	Stnd. Dev.	Range
10	7.6	±6.4	2.0-25.0	12.8	±9.9	2.0-40.5	6.7	±4.4	0.0-13.5
20	15.5	±8.6	1.0-23.0	19.2	±8.4	5.5-34.5	7.8	±6.1	0.5-19.0
30	20.7	±7.6	4.0-32.0	17.8	±6.0	10.5-30.5	6.2	±3.3	1.5-14.5
45	15.9	±6.7	2.5-25.5	15.6	±5.8	5.2-28.5	5.6	±3.3	1.0-11.5
60	11.8	±6.9	0.8-23.5	14.2	±5.7	2.0-27.0	3.4	±3.6	0.0-11.0

tients receiving 0.25 ml. per kg. The data in the tables and plotted on the curve represent the increase in alcohol level above normal rather than the absolute level. Note the close proximity of the two curves for the treated and untreated at the high dose. Testing of the mean values at each time interval at which blood was drawn showed no significant difference.

Acetaldehyde levels

The mean normal acetaldehyde level for the 10 untreated patients was 92 ± 38 micrograms per cent. For the 51 treated patients the value was 281 ± 187 micrograms per cent. These two means are significantly different having a p value of 0.006. This difference indicates that Antabus has a definite effect on the normal resting acetaldehyde level in man. This is in contrast to the report of Jacobsen (13) who found that treatment with Antabus alone caused no increase in the resting acetaldehyde value in rabbits.

The increase in blood acetaldehyde following alcohol ingestion is much more marked when the patient is treated with Antabus than when untreated (Table II and Figure 2). This observation confirms the finding that Antabus causes an accumulation of acetaldehyde in the blood. Figure 2, curve A shows the increase in aldehyde over normal plotted against time after 0.5 ml. per kg. of alcoholic beverage in untreated patients. Curve B is the data for the treated patients who received the same dosage. Curve C is the increase in acetaldehyde for Antabus treated patients who received 0.25 ml. per kg. Note that in the treated patients receiving the low dose, the increase in acetaldehyde was much greater than one-half that in the patients receiving twice the dose.

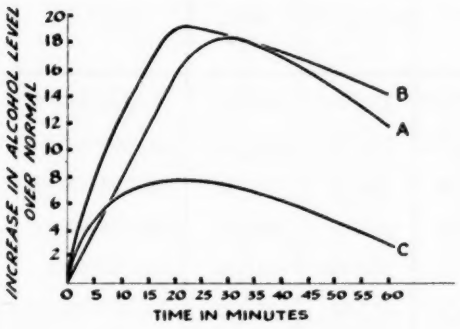

FIG. 1

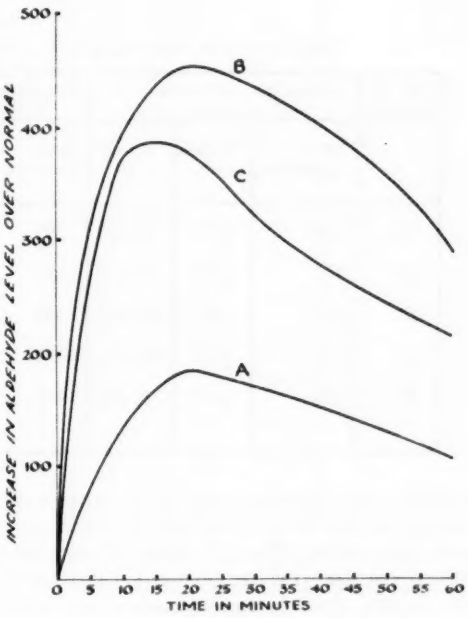

FIG. 2

While the peak of the mean values represented an increase of 450 micrograms per cent, it is of interest to note that some patients obtained much greater increases. Six patients had an increase in blood acetaldehyde of over 1,000 micrograms per cent; 15 of the 51 patients had an increase of over 600 micrograms per cent at some time during the first hour after the test drink.

Blood pressure

The most striking objective effect of an alcohol-Antabus reaction is the accompanying fall in blood pressure. The maximum fall occurs between 30 and 40 minutes after the ingestion of the alcohol though there is considerable variation. At the high dose the mean blood pressure of 31 patients fell from 132/84 to 107/55 in 40 minutes. This is a highly significant change. "T" testing gives a probability of less than 0.001 for both diastolic and systolic pressures. In some cases the fall in pressure was dramatic. Of a total of 156 blood

TABLE II

Mean increase in acetaldehyde levels of treated and untreated patients after test drink

Time in Minutes	Untreated Patients 0.5 ml./kg.			Treated Patients 0.5 ml./kg.			Treated Patients 0.25 ml./kg.		
	Mean Increase	Stnd. Dev.	Range	Mean Increase	Stnd. Dev.	Range	Mean Increase	Stnd. Dev.	Range
10	136	±66	30-237	399	±318	0-1112	373	±330	0-1325
20	185	±115	25-470	453	±292	22-1338	377	±369	0-1680
30	167	±67	105-326	421	±342	0-1202	319	±220	0-840
45	175	±118	98-373	399	±278	0-1185	262	±171	0-665
60	156	±167	0-320	275	±233	0-937	213	±194	0-660

pressure measurements made on 31 patients receiving the high dosage of alcohol, 24 systolic pressures were below 100 and 11 below 80 some time during the test. Of the same group there were 46 diastolic blood pressure measurements below 60 and 15 below 40. Six patients had blood pressures below 80/40 at some time during the first hour after the test drink.

For the low dose the drop was not significant ($p = 0.45$), the mean maximum drop being from 130/86 to 124/74 in 40 minutes. In addition, of 135 blood pressure determinations made on patients receiving the low dosage only one systolic reading dropped below 100 and five diastolic readings below 60 (Table III).

No significant change was noted in the blood pressure of patients taking alcohol alone and untreated with Antabus.

There was no significant change in pulse pressure at either the high or low dose when the patients were considered as a group. However, eight patients at the high dose, and three at the low dose had a large increase in pulse pressure in the first 10 minutes after the test drink. By 20 minutes

TABLE III

Mean blood pressures for both groups of treated patients at various time intervals

Time In Minutes	Treated Patients 0.5 ml./kg.			Treated Patients 0.25 ml./kg.		
	Mean B. P.	Stnd. Dev.	Range	Mean B. P.	Stnd. Dev.	Range
Before	132/84	±10/±8	166/110-114/70	130/86	±14/±9	175/108-110/70
10	129/74	±10/±12	154/92-114/50	128/82	±16/±12	165/106-106/62
20	117/64	±19/±16	154/88-60/30	126/76	±20/±17	184/108-102/64
30	112/61	±21/±17	150/96-66/22	123/75	±21/±16	180/102-90/50
45	114/65	±17/±13	146/94-68/44	120/76	±11/±11	140/94-96/56
60	108/65	±19/±16	140/94-72/34	125/82	±15/±10	162/108-102/70

TABLE IV

Mean pulse rates for treated patients at both dose levels

Time in Minutes		Treated Patients 0.5 ml./kg.		Treated Patients 0.25 ml./kg.		
	Mean Rate	Stnd. Dev.	Range	Mean Rate	Stnd. Dev.	Range
Before	80	±13	52-112	80	±13	52-112
10	99	±19	54-128	102	±16	82-142
20	106	±17	82-150	104	±13	82-136
30	100	±16	76-142	100	±15	76-130
45	94	±16	66-118	97	±15	80-126
60	94	±13	64-118	89	±13	72-120

the pulse pressures were again in the normal range. All the patients whose pressures fell below 80/40 had a significant narrowing of pulse pressure when compared with the mean normal pressure. This, however, would be expected for a shock-like state.

Pulse rate

An increase in pulse rate was noted in every patient undergoing a clinically detectable alcohol-Antabus reaction. The pulse rate reached a maximum at about 20 minutes and was slow in returning to normal. A rather interesting finding was that in five of the six patients whose blood pressures fell below 80/40, pulse rates were below 95 in spite of the fact that their rates had been higher before the fall in blood pressure was marked. Also unusual was the fact that there was no difference in the mean increase in pulse rate in those patients receiving the high as contrasted to those receiving the low dosage in spite of the fact that the

TABLE V

Mean respiratory rates for both groups of treated patients at various time intervals

Time In Minutes	Treated Patients 0.5 ml./kg.			Treated Patients 0.25 ml./kg.		
	Mean Rate	Stnd. Dev.	Range	Mean Rate	Stnd. Dev.	Range
Before	18	±2	12-24	18	±2	12-24
10	21	±4	10-28	22	±5	16-32
20	22	±4	16-32	23	±4	16-32
30	22	±4	16-36	22	±4	16-34
45	20	±3	12-28	21	±5	16-34
60	20	±3	16-26	19	±3	16-30

overall reactions were more severe in the patients receiving the high dosage. Pulse rates of 120 or more occurred in nine out of 33 patients given the high dose, and five out of 22 patients given the low dose. Five patients (three given the high and two given the low dosage) had a pulse rate in excess of 140 per minute. Table IV gives data on mean pulse rates at various time intervals.

Respiratory rate

In this study the most striking respiratory change was the increase in depth of respiration

TABLE VI

Table of data concerning signs and symptoms

High Dose in 44 Patients

SIGN OR SYMPTOM	MEAN ONSET	STND. DEV.	MEAN PEAK	STND. DEV.	MEAN RATING *	NUMBER FOUND IN	PER-CENT
Conjunctival Injection	10	±5	36	±13	2.2	39	89
Flushing	7	±3	25	±10	2.1	42	95
Feeling of Heat	5	±2	23	±8	1.9	42	95
Throbbing	16	±8	29	±9	2.3	16	36
Dyspnea	12	±7	25	±9	1.9	25	57
Palpitation	13	±6	28	±8	2.1	24	55
Dizziness	18	±10	32	±12	1.8	15	34
Nausea	36	±22	48	±32	2.0	11	25
Vomiting	—	—	—	—	2.2	5	11
Cold Shiver	—	—	—	—	2.1	8	18
Sweating	—	—	—	—	1.8	6	14
Abnormal Odor (Breath)	12	±7	43	±16	2.1	30	68
General Indisposition	31	±15	45	±22	2.2	17	39
Sleepiness	33	±18	56	±24	1.8	25	57

Low Dose in 29 Patients

SIGN OR SYMPTOM	MEAN ONSET	STND. DEV.	MEAN PEAK	STND. DEV.	MEAN RATING *	NUMBER FOUND IN	PER-CENT
Conjunctival Injection	10	±4	31	±12	1.6	26	90
Flushing	7	±6	23	±10	1.8	27	93
Feeling of Heat	6	±3	23	±12	1.6	24	83
Throbbing	11	±4	25	±8	1.5	7	24
Dyspnea	11	±5	22	±6	1.6	14	48
Palpitation	14	±7	25	±9	1.5	16	55
Dizziness	22	±18	34	±19	1.4	8	28
Nausea	—	—	—	—	1.5	4	14
Vomiting	—	—	—	—	—	0	—
Cold Shiver	—	—	—	—	2.0	1	—
Sweating	—	—	—	—	1.7	4	14
Abnormal Odor (Breath)	12	±6	26	±8	1.6	14	48
General Indisposition	19	±11	38	±12	1.7	7	24
Sleepiness	31	±17	41	±18	1.3	16	55

* The mean rating is the mean of the intensity rating with 1 being considered mild, 2 moderate, and 3 severe. These are without a doubt highly subjective data but nonetheless indicate how the investigators felt about the relative influence of the two doses on any particular sign or symptom.

rather than rate. Nonetheless the mean change in the rate from 18 to 23 a minute for the "low dose" group and 18 to 22 for the "high dose" group was highly significant. Upon "t" testing the value for p was less than 0.001. In many cases the patient's subjective complaint of dyspnea was all out of proportion to the change in depth and rate indicating that the phenomenon was more than simple respiratory stimulation.

There were only three patients in the entire group at both dosage levels who had a maximum increase in respiration of more than 12 a minute and only one of those doubled his rate (16 to 34 a minute). Table V illustrates the mean results of respiratory changes in the patients studied.

Signs and symptoms

For the sake of brevity these data will be presented in tabular form with only a few comments here.

The conjunctival injection, blushing, feeling of heat, throbbing, and palpitation (Table VI, all signs and symptoms of cardiovascular origin) have a mean onset between five and 16 minutes and a mean peak between 23 and 36 minutes. If conjunctival injection is excluded, then the mean peak is between 23 and 29 minutes and this is irrespective of dose. This correlates with the onset and peak of pulse rate changes.

Patients frequently complained of dyspnea, tightness of the throat, and of increased expiratory effort. Despite these complaints, the change in respiratory rate was not striking.

Nausea, vomiting, cold shivers, and sweating were so infrequent and the time of onset and peak so variable that mean values were not computed. Further, these four symptoms so frequently have a psychosomatic origin that it is questionable whether their origin is entirely "organic." Vomiting did not occur at all in patients receiving the low dosage.

General indisposition was rated as mild when the patient was unable to walk around, moderate when the reclining position was necessary, and severe when the patient was in a near shock-like state.

Sleepiness became a problem for out-patients. Some of these patients could not stay awake even with effort. Many had to be kept in the hospital from one to four hours after the test period was over before they could be allowed to drive their cars or ride on public transportation.

Several symptoms that were not listed on the check sheet occurred frequently. Six of the patients had tremors that resembled those seen in hypocalcemic tetany. Four patients complained of a pain and tightness in the chest similar to angina pectoris. This symptom was considered serious enough in one patient to warrant cessation of Antabus therapy. Incidentally, this patient had an abnormal EKG when taken during the alcohol-Antabus reaction.

Several patients complained of a metallic taste in the mouth similar to the taste of tarnished silver. This raised the possibility of salivary excretion of Antabus or an Antabus breakdown product. Numerous other complaints occurred but not often enough to accredit them to the alcohol-Antabus reaction.

Itching, expressions of fear, and convulsions did not occur in this group of patients though they were looked for. However, two patients treated with Antabus in a later series but not included in this group had convulsions during a reaction (see Table VI).

DISCUSSION

Antabus apparently has no effect on the rate of disappearance of alcohol. Figure 1 shows the normal and control curves to be very similar. This lack of difference is statistically significant even though only 10 controls are compared with 51 experimental subjects.

An increased alcohol level is necessary for the reaction to take place. In three of the patients studied, no increase in alcohol level was found after the test drink. In none of the three was there any clinical reaction whatever. It is difficult to explain why there was no increase in the blood alcohol level in these patients since they were all presumably in the fasting state. Subsequent tests a week later with double the dose of alcohol in each of these patients produced a definite reaction. The blood alcohol increase was then appreciable.

For the most part, the higher the alcohol level the more severe the reaction (up to 49 mg. per cent, our highest level); however, there were frequent notable exceptions. For example, one patient who on the first test had a 34 mg. per cent increase in blood alcohol showed only a mild reac-

tion. The following week this patient was retested at the lower dose. He had an 11 mg. per cent increase in blood level and a severe reaction. A possible explanation for this discrepancy may be that there was an insufficient quantity of Antabus in the tissues at the time of the first test.

Since the rate of alcohol disappearance is not affected, it is probable that the patient's reaction is due to an accumulation of acetaldehyde in the tissues as a result of inhibition of the acetaldehyde oxidation. Comparison of the three curves in Figure 2 illustrates the effect Antabus has on blood acetaldehyde levels. Note that the difference between the acetaldehyde curves at the low and high alcohol dose is not great and further that the mean values of increase in acetaldehyde level for the low dose is considerably more than half that for the high dose at all time intervals. This suggests that at some alcohol blood levels, in spite of Antabus, the acetaldehyde level will have a maximum increase and that higher alcohol doses will have no further effect. This suggestion is compatible with Kjeldgaard's (8) conclusion that the inhibition of aldehyde oxidase by Antabus is competitive.

The increase in the normal blood acetaldehyde (before alcohol ingestion) in Antabus treated as compared to untreated persons was an unexpected finding in light of Jacobsen's (13) statement that there was no increase in the blood acetaldehyde level in treated rabbits. From this it may be assumed that this response is a species difference.

The observations that the alcohol levels and most of the subjective and objective signs and symptoms reach a peak at the same time as the acetaldehyde levels lend further support to the theory that the acetaldehyde is the toxic agent. In this study only two patients became quite ill early (within 20 minutes) without an appreciable rise in acetaldehyde level. The reaction picture in these patients was somewhat different from that usually observed. Headache, nausea and malaise were outstanding symptoms and occurred early. The cardiovascular complaints occurred after 45 minutes and were accompanied by a delayed rise in acetaldehyde levels. The early complaints in these patients may be evidence of a toxic factor other than acetaldehyde.

Further evidence of the presence of other toxic factors is suggested by the following. Some 15 patients phoned several hours after the completion of the test and complained that they had the worst hangover of their lives. All of these patients' blood alcohol and acetaldehyde levels had returned to normal before they were permitted to leave. All vigorously denied subsequent drinking.

One of the most dangerous effects of the reaction was the fall in blood pressure. Six patients had blood pressure drops to shock levels. One patient not included in this report had an unobtainable pressure for 15 minutes. In addition, five of these six patients had a low pulse rate accompanying the low blood pressure, indicating myocardial dysfunction. This supports Handovsky's (14) finding that acetaldehyde has a direct toxic myocardial action. Such patients responded well to oxygen, rest and intravenous ephedrine. One patient in the series who drank in spite of warning suffered a myocardial infarction (15). This might have been related to the fall in blood pressure and the direct myocardial toxicity of acetaldehyde.

The disproportionately large number of complaints of dyspnea as compared to the small change in rate and amplitude of breathing, suggests that acetaldehyde in addition to the respiratory stimulating effect, adversely affects the respiratory apparatus. It has been the experience of two of the authors (Hine and Burbridge) that acetaldehyde given in large doses to rabbits and rats intravenously will cause an acutely inflamed lung, with small punctate hemorrhages. This would support the idea of direct toxicity of acetaldehyde to the respiratory apparatus.

Cardiac arrhythmias, electrocardiographic evidence of myocardial ischemia and even myocardial infarction may occur during the Antabus-alcohol reaction (15).

Antabus itself is apparently a relatively safe drug, and in this study there has been no indication of damage to any organ due to its chronic ingestion in the dosages used. However, the clinical observations made during this study indicate that the use of Antabus in treating or testing the problem drinker is accompanied by definite risks. The reaction which occurs following the ingestion of alcohol by a patient who is on Antabus therapy is severe and may occasionally lead to severe injury and even death. We strongly recommend the careful observation of the cardiovascular changes which accompany the reaction during a test drink. The physician should be prepared to ad-

minister emergency supportive therapy such as administration of oxygen and placement in the shock position if required. The patient who is chosen for Antabus therapy should be carefully warned concerning the severity of the reaction which may occur if he drinks even small quantities of alcohol.

SUMMARY AND CONCLUSIONS

1. A study was made of 51 problem drinkers following a test drink of alcohol prior to and after Antabus therapy with attention directed to the relationship among the following: blood levels of alcohol and acetaldehyde, clinical symptoms, changes in pulse, respiration and blood pressure.

2. Antabus was not found to alter normal blood alcohol levels. Neither did the compound appear to affect either the rate of absorption or metabolism of the ethanol itself.

3. Antabus significantly increased the normal resting acetaldehyde level in man.

4. There was a very significant increase in blood acetaldehyde in the Antabus treated subject as compared to the untreated patient after the ingestion of alcohol.

5. Evidence is presented to support the view that acetaldehyde is the toxic agent responsible for the most serious consequences of the alcohol-Antabus reaction.

6. Serious decreases in arterial pressure as a result of the alcohol-Antabus reaction are reported.

7. Pulse rate and respiratory rate increased significantly during the alcohol-Antabus reaction.

8. A detailed picture of the clinical symptomatology is presented.

9. In light of the often dangerous cardiovascular effects of Antabus, recommendations for the careful use of the drug were made.

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REVERSAL OF DIURNAL VARIATION IN RENAL FUNCTION IN CASES OF CIRRHOSIS WITH ASCITES [1, 2]

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The mechanism for control of diurnal rhythm of water and electrolyte excretion remains obscure, though the fact that such a cycle occurs was reported by Quincke (1) in 1877 who pointed out that urinary output was appreciably higher during the day in contrast to night. Campbell and Webster (2, 3) observed that the excretion of chlorides and urea was also higher during the day, though creatinine excretion remained more or less at the same level. Subsequent studies (Table I) by Kleitman (4), Simpson (5, 6), Norn (7), Manchester (8), Gerritzen (9), Brod (10), and Sirota, Baldwin, and Villarreal (11) confirmed the observations that urine flow and excretion of certain electrolytes decreased during the hours of deepest sleep in normal individuals.

This rhythm was not reversed when normal subjects slept in the daytime and took food and worked at night as reported by Campbell and Webster (2, 3), and confirmed in studies of a night watchman by Jores (12). Gerritzen (13) studied the effect of exposure of four subjects to artificial daylight during the night, and darkness during the day. In three subjects duplication of the rhythm in water and chloride excretion resulted, with two maxima occurring at noon and at midnight. In the fourth subject the rhythm was completely reversed. Borst and de Vries (14) have pointed out the difficulty in evaluating the effect of daylight and of waking on diurnal rhythm, though in some of their subjects the response to the stimulus of daylight was prompt. Jores (12) reported the relative difficulty in reversing a rhythm by a reversal of routine, and this was further supported by Joslings' (15) observation that a subject on a boat going from east to west showed a peak urinary excretion one hour earlier every day, the peak remaining at the same hour on local time. Addis and associates (16) reported nocturnal depression of endogenous creatinine clearances.

On the basis of U/P inulin ratios and creatinine clearance data, Sirota, Baldwin, and Villarreal (11) attributed the decrease in urine flow during sleep almost wholly to water reabsorption. The mechanisms of increased tubular reabsorption of water at night were not elucidated and significant diurnal variations in effective renal plasma flow were not noted (11).

Reports of a reversal of renal function (Table II) began with Wilson's (17) observation that the day and night rates of excretion of water and solids were approximately the same in the presence of debility, and especially so in cardiac or renal disease. Quincke (18) confirmed these observations several years later, noting that patients with heart or kidney disease often had a peak of diuresis during the night. Fishberg (19) attributed the nocturnal increase in urine flow in patients with nephritis and congestive heart failure to decreased renal venous pressure and increased cardiac output in the prone position. Subsequent work by Brod and Fejfar (20) and by Baldwin, Sirota, and Villarreal (21), employing clearance techniques, revealed an increased glomerular filtration rate and decreased tubular reabsorption of water during the hours of sleep in patients with congestive heart failure. In the absence of peripheral edema, or during active diuresis, no reversal of the diurnal pattern was observed (21). According to Brod and Fejfar (20), nocturia was not associated with an increase in cardiac output. Since nocturnal diuresis was always associated with an increase in

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[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

TABLE I

Studies of diurnal variations of renal function in normal individuals

Investigator	Renal Functions Studied		
	No Diurnal Change	Nocturnal Depression Observed	Attributed to
Quincke (1) (1877)	——	Urine flow	——
Campbell (2,3) (1921-'22)	Creatinine excretion	Urine flow, excr. urea, Cl.	——
Kleitman (4) (1923)	Nitrogen or creatinine excr.	Urine flow, excr. of phosphates, acids and Cl.	Decr. blood pressure.
Simpson (5,6) (1924,1926)	——	Urine flow, phosphate and Cl. excr., urine pH.	Acidosis of sleep with intracellular shift of chloride and water.
Norn (7) (1929)	——	Urine flow, excr. of Na., K., Cl.	Diminished activity
Manchester (8)* (1933)	Excr. of Ca., Mg., (Data on phosphate, sulfate, ammonia excr. & titratable acidity were equivocal).	Urine flow, excr. of Na., K., Cl.	Related to sleep
Gerritsen (9) (1940)	——	Urine flow, excr. urea, Cl.	Rhythmic organ function and position change.
Brod (10) (1946)	——	Urine flow, endog. creat. clear.	——
Sirota (11) (1950)	PAH clear.	Urine flow, inulin clear.	Variations in tubular reabsorption.
Borst (14) (1950)	Excr. urea, creatinine.	Urine flow, excr. Na., Cl., K.	Variations in tubular reabsorption.
Addis (16) (1951)	——	Endog. creat. clear.	Relaxation of vasc. tension with drop in filt. press.

* Data on two epileptic children on graded activity.

renal blood flow and a decrease in filtration fraction, they attributed the nocturia to the increased flow of blood to the kidneys during the period of complete rest.

Previous reports of renal function in cirrhosis (Table III) are conflicting. Leslie, Johnson, and Ralli (22) reported a depression of renal hemodynamics in cirrhosis. They observed higher rates of glomerular filtration and effective renal plasma flow in patients without ascites or during active diuresis than in the presence of actively reaccumulating ascites. Patek and associates (23), and more recently Goodyer, Relman, Lawrason, and Epstein (24) observed that mean glomerular filtration rates and effective renal plasma flows were within normal limits in this disease. Farnsworth and Krakusin (25) showed that the electrolyte excretion in patients with hepatic cirrhosis and ascites resembled that seen in cardiac failure. More recently, Goldman (26) observed a nocturnal diuresis of water and sodium in patients with congestive heart failure, glomerulonephritis, and cirrhosis of the liver. The nocturia and increased nocturnal chloride excretion in patients with cirrhosis reported by Smits (27) was attributed by Borst and de Vries (14) to semi-recumbent position at night.

During the course of water partition studies in cirrhotics with ascites, at which time renal clearances were performed, a reversal of diurnal rhythm in renal function was noted. An abstract of these observations has been published elsewhere (28).

TABLE II

Studies of diurnal variations of renal function in certain pathologic states

Investigator	Pathologic State		Renal Function Variation		
			Studied	Observed	Attributed to
Quincke[18] (1893)	Nephritis Congestive heart failure		Water excretion	Nocturia	-----
Wilson[17] (1889)	Nephritis Congestive heart failure		Water and solid excretion	Nocturia, incr. solid excr.	-----
Fishberg[19] (1939)	Nephritis Congestive heart failure		Water and electrolyte excr.	Nocturia	Decr. renal venous press., incr. cardiac output in prone pos.
Brod[10] (1946)	Glomerulonephritis		Creatinine clear.	Nocturia, decr. H_2O reabsorb.	-----
	Congestive heart failure		U/P ratios.	Nocturia, incr. GFR, decr. H_2O reabsorb.	-----
Baldwin[21] et al (1950)	Congestive heart failure	Edematous	Inulin and creatinine clear., U/P ratios, excr. Na.	Nocturia, incr. GFR, incr. Na excr.	Nocturia not related to level of GFR.
		Edema-free		Decr. urine flow, decr. GFR, decr. Na excr. (at night)	-----
Brod & Feijfar[20] (1950)	Congestive heart failure		Inulin, PAH clear. cardiac output.	Nocturia, incr. GFR, incr. ERPF, decr. F.F., No change in cardiac output	Incr. of blood flow to kidneys with diminished activity.
Smits[27] 1950)	Cirrhosis Congestive heart failure		Water and Cl excr.	Nocturia, incr. Cl excr.	Semi-recumbent position at night.
Goldman[26] (1951)	Congestive heart failure Glomerulonephritis Cirrhosis		Na, K, H_2O excr.	Nocturia, incr. Na excr., No change K excr.	Unknown humoral factors

The present paper presents further data on diurnal variations in renal hemodynamics in normal subjects and cases of cirrhosis.

METHODS

The diagnosis of Laennec's cirrhosis was made by the usual clinical and laboratory methods in the patients studied. Each of these patients had been hospitalized one or more times previously at Hines Veterans Administration Hospital, at which time the diagnosis of cirrhosis had been established. Patients selected for this study manifested no clinical evidence of cardiovascular renal disease as judged by past history, physical findings, electrocardiographic and laboratory findings. This group had in common the presence of reaccumulating ascites (Table IV), which required repeated hospital admissions and paracenteses. Normal subjects were healthy adult males who were either awaiting elective surgery or convalescing from minor surgical procedures. The subjects were allowed food and liquid *ad libitum* but were kept recumbent in bed throughout the experimental procedure. No preliminary hydration was employed. The clearance of inulin was used to estimate glomerular filtration rate (GFR) and that of para-aminohippurate to estimate effective renal plasma flow (ERPF). A priming injection of inulin and para-aminohippurate was followed by a sustaining infusion delivered at a constant rate by a pump over a 24 hour period. All solutions were infused into an antecubital vein by means of nylon tubing which permitted the patient to move about freely in bed without fear of subcutaneous extravasation of the infusate (29). Urine samples were collected by an indwelling multi-eyed catheter. At the conclusion of each clearance period, the bladder was rinsed with 60 cc. of saline to insure quantitative recovery of all urine. The spot clearance periods were 20 to 30 minutes in duration and in addition a concurrent 24 hour period was run on each patient. Two consecutive periods were run during the morning hours (9 a.m. to 12 noon) and subsequently during the late evening or early morning hours (11 p.m. to 3 a.m.). Blood samples were collected in heparinized tubes at the mid-point of each clearance period by means of an indwelling 17 gauge needle. Every attempt was made to avoid psychic or physical trauma to the patient and no

TABLE III

Summary of literature: renal hemodynamics in cirrhosis and ascites

Investigator	Number of Patients	Glomerular Filtration Rate (cc./min.)	Effective Renal Plasma Flow (cc./min.)
Patek et al * (23) (1948)	3	135 * ↓ (91 - 172)	581 ↓ (434 - 709)
Farnsworth and Krakusin (25) (1948)	2	68 ** ‡ (61 - 74)	446 ‡ (415 - 478)
Leslie (22) (1949)	5	69 * ‡	371 ‡
Goodyer et al (24) (1950)	6	120 ** ↓ (60 - 124)	471 ↓ (298 - 647)

* Inulin clearance

** Mannitol clearance

↓ Corrected to 1.73 m^2.

‡ Uncorrected data

Note: Max. and min. values appear in parenthesis.

additional venapunctures or urethral instrumentation were performed after the experiment had begun. Inulin was determined by Harrison's (30) modification of the method of Alving, Rubin, and Miller (31). Para-aminohippurate was determined by the method of Smith and associates (32) as described by Goldring and Chasis (33).

Measurements of extracellular volume (employing a continuous infusion of mannitol over eight hours) were usually made concurrently with the day time clearance periods. Since the mannitol infusions produced a mild diuresis and as such affected urine flow values and inulin U/P ratios, these data were omitted in a consideration of diurnal variations in renal function. At plasma levels (50–150 mg. %) used to measure extracellular volume, mannitol is known to have no affect on either filtration rate or renal plasma flow (33, 34).

RESULTS

Table V summarizes day, night, and 24 hour GFR data in normal and cirrhotic subjects. Four out of the five normal subjects showed a definite

TABLE IV

Estimated severity of liver disease in patients studied for diurnal variations in renal hemodynamics

Patient	Age	Severity of Liver Dysfunction		
		Number of * Admissions	History of Jaundice	Degree of Ascites ↓
1. J. S.	61	3	Yes	+++
2. V. G.	53	2	Yes	+
3. K. N.	49	3	Yes	++++
4. J. K.	40	2	Yes	++
5. C. R.	57	3	No	+
6. C. R.	53	2	Yes	++
7. C. N.	42	2	Yes	+
8. W. B.	41	2	Yes	++

* Hospital admissions for liver disease.

↓ Arbitrary quantitation of severity of ascites from ↓ thru +++

TABLE V

Diurnal variations in glomerular filtration rates in normal subjects and in cases of cirrhosis with ascites

SUBJECTS	GLOMERULAR FILTRATION RATE cc./min./1.73m²				24 Hr. G.F.R. cc./min./1.73m²
	Day	Night	Difference	% Change	
Normal Subjects					
1. K.G.	119.0	113.0	-6.0	-5.1	124.2
2. (D.M.)	(113.7)	(127.0)	(+13.3)	(+11.7)	(122.0)
3. J.M.	110.0	105.7	-4.3	-3.9	103.9
4. R.N.	140.0	119.6	-20.4	-14.3	126.3
5. V.S.	131.2	120.0	-11.2	-8.5	127.2
$\bar{x}$	126.9	117.0	-5.7	-4.0	120.7
s	±13.6	±8.0	±12.3	±6.7	±9.6
P	-	-	0.2	-	-
Patients With Cirrhosis					
1. J.S.	128.2	156.0	+27.8	+21.6	162.0
2. V.G.	126.0	139.5	+13.5	+10.7	160.6
3. N.N.	95.5	122.6	+27.1	+28.5	118.9
4. J.K.	109.3	116.5	+ 7.2	+ 6.6	130.4
5. G.R.	127.1	154.5	+17.4	+13.7	150.2
6. C.R.	82.9	99.1	+16.2	+19.6	88.0
7. C.N.	74.4	90.6	+16.2	+21.8	84.5
8. W.B.	90.1	108.6	+18.5	+20.6	104.0
$\bar{x}$	104.2	123.4	18.0	17.9	124.8
s	±21.4	±24.6	±6.8	±7.1	±31.1
P	-	-	0.01	-	-

$\bar{x}$ = mean; s = standard deviation; P = probability value

TABLE VI

Diurnal variations in effective renal plasma flow in normal subjects and in cases of cirrhosis with ascites

Subjects	Effective Renal Plasma Flow (ERPF) cc./1.73m²				24 Hr. ERPF (cc./1.73m²)
	Day	Night	Difference	% Change	
Normal Subjects					
1. K.G.	713	631	-82	-11	691
2. D.M.	1130	1161	+31	+3	1160
3. J.M.	608	690	+82	+13	634
4. R.N.	1040	1380	+340	+33	1150
5. V.S.	806	627	-179	-22	735
$\bar{x}$	859	898	+38	+3	875
s	±222	±267	±196	±21	±262
P	—	—	0.5	—	—
Patients With Cirrhosis					
1. J.K.	292	514	+222	+76	672
2. G.R.	898	1212	+314	+35	982
3. C.R.	547	639	+92	+17	580
4. C.N.	354	594	+240	+68	445
5. W.B.	717	786	+69	+9.6	723
$\bar{x}$	562	749	187	41.1	680
s	±252	±277	±104	±29.8	±199
P	—	—	0.04	—	—

$\bar{x}$ = mean; s = standard deviation; P = probability value

TABLE VII

Diurnal variations in filtration fraction in normal subjects and in cases of cirrhosis with ascites

Subjects	Filtration Fraction ($C_{1/CPAH}$)				24 Hr. F.F.
	Day	Night	Difference	% Change	
Normal Subjects					
1. K.G.	16.6	17.8	+1.2	+7	17.9
2. D.M.	10.1	10.8	+0.7	+1	10.3
3. J.M.	18.1	15.4	-2.7	-15	16.7
4. R.N.	13.5	8.7	-4.8	-35	10.9
5. V.S.	16.3	19.1	+2.8	+17	17.3
$\bar{x}$	14.9	14.3	-0.5	-3.0	14.6
s	±3.1	±4.4	±2.8	±20.0	±3.6
Patients with Cirrhosis					
1. J.K.	37.4	22.4	-15.4	-42	19.4
2. G.R.	14.2	12.8	-1.4	-9	15.3
3. C.R.	15.2	15.5	+0.3	+2	15.1
4. C.N.	21.0	15.3	-5.7	-27	19.0
5. W.B.	12.6	13.8	+1.2	+9.5	14.4
$\bar{x}$	20.1	16.0	-4.2	-13.3	16.6
s	±10.2	±3.8	±7.0	±21.1	±2.4
P	--	--	0.2	--	--

$\bar{x}$ - mean; s - standard deviation; P - probability value

tendency toward a decreased GFR during the night. However, the mean day-night difference was not statistically significant. All of the cirrhotic patients showed a nocturnal increase in GFR. The night values averaged 123.4 ± 24.6 cc./min. whereas the mean for the day period was 104.2 ± 21.4 cc./min. The mean difference of 18.0 cc./min. was highly significant with a P value of less than 0.01.

Data on diurnal variations in ERPF are shown in Table VI. In normal subjects there was no consistent change in nocturnal ERPF. Three out of five subjects showed a moderate increase whereas two showed a decrease in ERPF during the night. In striking contrast, all five of the cirrhotic patients infused with PAH showed a significant nocturnal increase in ERPF with a mean increase of 187 ± 104 cc./min. (P = 0.04).

Filtration fractions (FF) in normal and cirrhotic subjects are presented in Table VII. In normal subjects there was no consistent diurnal change in FF. Although the mean difference between the day and night FF in cirrhotic subjects was not statistically significant, it should be noted that there was a marked nocturnal fall in FF in two patients in whom the day FF values were abnormally high. On the average there was a tendency for a nocturnal decrease in FF in cirrhotic patients.

Data comparing 24 hour clearance values with those obtained during spot clearance periods are shown in Tables V, VI, and VII. In normal subjects, the 24 hour GFR calculated from the 24 hour clearance of inulin averaged 120.7 ± 9.6 cc./min., a value which fell between the day and night clearances. In cirrhotic subjects, the mean 24 hour GFR of 124.8 ± 31.1 cc./min. was actually greater than either the average day or night values. In normal subjects the 24 hour ERPF and FF values fell between those of the day and night clearance periods, whereas in cirrhotic patients these data more closely approximated those of the night clearance periods.

DISCUSSION

In normal subjects there was a tendency toward a nocturnal decrease in GFR without consistent changes in ERPF. These findings agree with those reported by Sirota, Baldwin, and Villarreal (11). In contrast to the findings in normal subjects, patients with cirrhosis and ascites showed a significant nocturnal increase in GFR and ERPF.

Both the normal subjects and cirrhotic patients were allowed food and water *ad libitum* during the renal tests. It has been shown that variations in urine flow by the administration of water with resultant flows of 1.0 up to 12.0 cc./min. had no effect on renal hemodynamics (35), and as such would rule against any influences due to the ingestion of fluids during the waking hours. All patients were maintained on high protein, high carbohydrate, low fat diets. High protein diets have been shown to increase filtration rate and renal plasma flow when fed over a 102 day period in normal subjects (36). Little information is available in the literature on the acute effects of meals on renal hemodynamics. A large beef steak meal as compared with a light breakfast was reported to have no effect on the renal plasma flow in one subject although it slightly increased the filtration rate (37). The nocturnal reversal in diurnal rhythm in cirrhotic patients would thus not seem to be related to either food or water intake during the waking hours.

The reversal of diurnal rhythm in renal hemodynamics observed in our patients with cirrhosis and ascites was similar to that reported by Baldwin, Sirota, and Villarreal (21) and Brod (10, 20) in patients with congestive heart failure. Brod and Fejfar (20) noted that the spontaneous nocturnal diuresis of cardiac patients was always accompanied by an increase in ERPF and a fall in filtration fraction. In our series of cirrhotic patients, those with abnormally high day FF values showed a similar nocturnal fall in FF. Brod and Fejfar theorized that the nocturnal improvement in renal blood flow was the result of redistribution of body water and blood flow in association with a decreased metabolic demand by muscles and viscera during the night hours.

Goldman (26) recently reported a nocturnal diuresis and natriuresis in patients with cirrhosis of the liver and ascites, in cases of congestive heart failure, and in two out of five patients with degenerative glomerulonephritis. He postulated that the nocturnal natriuresis might depend on variations in tubular metabolism controlled by a humoral agent which perhaps would be affected by the functional state of the liver.

Antidiuretic substances have previously been reported in the urine of some patients with congestive heart failure (38), cirrhosis (39), acute hepatitis (40), nephrotic edema (41), and hypertension (42). Although a diurnal variation in the secretion of ADH by the posterior pituitary could account for the nocturia in these conditions, it would fail to explain the changes in glomerular filtration rate and renal plasma flow. It has been reported that the infusion of physiologic doses of pitressin had no significant effect on renal hemodynamics in man (43).

Farnsworth and Krakusin (25) noted that in both cirrhosis with ascites and in congestive heart failure, the kidneys behaved as if the body were dehydrated. They submitted the hypothesis that a specific stimulus to salt and water retention was probably acting in both diseases. Deming and Luetscher (44) have reported an increase in excretion of desoxycorticosterone-like substances in edematous patients with heart failure or nephrosis. Evidence of increased activity of desoxycorticosterone-like hormones also has been reported in cirrhosis (45). In normal dogs, it has been shown that large doses of desoxycorticosterone expand the inulin space (46) and increase filtration rate and renal plasma flow (46, 47). It has recently been demonstrated that ACTH may effect an impressive diuresis and natriuresis in cases of nephrosis (48–51). It has been further shown by Rosenbaum, Davis, and Ferguson (52) that nocturnal oliguria and suppression of electrolyte excretion occurring in normal subjects could be eliminated or diminished as a result of cortisone therapy with an actual reversal of the rhythm. Recent work by Gaunt, Birnie and Eversole (53) has led to the theory of a reciprocal or antagonistic action between ADH and adrenal cortical hormones. It is entirely possible that the reversal of diurnal rhythm in renal function noted in various edematous states may be due to increased adrenal cortical activity during the night hours with relative neutralization of ADH effects.

In this series of eight patients with cirrhosis and ascites, four showed abnormally low day-time GFR values, whereas the rest showed rates within normal limits. Two of the four cirrhotic patients with depressed day-time filtration rates had 24 hour GFR values which were within normal limits. Two out of five patients showed an impairment in ERPF and an abnormal elevation of filtration fraction. These findings agree with those reported in the literature (22–25). It was difficult to cor-

relate any impairment of renal function with the severity of the cirrhosis, degree of ascites or with any past history of cardiovascular renal disease.

The importance of 24 hour measurement of glomerular filtration rate and renal plasma flow is pointed up by these studies in normal and cirrhotic subjects. In normal subjects, minimal diurnal variations did not result in significant differences between the 24 hour values and those obtained in spot clearance periods. In cirrhotics, however, spot clearance periods run during the day deviated markedly from 24 hour measurements of glomerular filtration rate and renal plasma flow. The mean 24 hour values in cirrhotic individuals more closely approximated the values obtained during the nocturnal clearance periods. These discrepancies are of particular importance in studies of disturbances in electrolyte excretion in edematous states.

SUMMARY

1). In a series of cases of cirrhosis with ascites a reversal of diurnal rhythm was noted with a nocturnal increase in glomerular filtration rate and effective renal plasma flow. Those patients with impaired renal flows showed a fall in FF as renal blood flow improved during the night. The possible participation of hormonal factors in the diurnal reversal is discussed.

2). Twenty-four hour measurements of renal hemodynamics were within normal limits in six out of eight patients with cirrhosis and ascites.

3). In patients with marked diurnal variations in renal hemodynamics, spot clearance periods run during the waking hours were appreciably lower than those obtained through the 24 hour clearance period. The importance of this fact in relation to electrolyte balance studies is emphasized.

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STUDIES IN GLYCINE-2-C^{14} METABOLISM IN MAN. II. TISSUE DISTRIBUTION [1]

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(Submitted for publication November 15, 1951; accepted January 8, 1952)

In a previous communication describing the pulmonary excretion of $C^{14}O_2$ in patients following the intravenous administration of 100 microcuries of glycine–2–C^{14} it was demonstrated that approximately 83 to 92 per cent was excreted via the lungs and that approximately 3 to 5 per cent was excreted within the urine in the first 10 days (1).[2] At that time preliminary autopsy data on one patient disclosed that about 10 per cent of the C^{14} was retained in compounds having a longer turnover time than the slowest component of excretion in the breath, namely the 7- to 14-day component. Since the publication of this paper three additional patients who were given C^{14} labeled glycine for the study of the life span of the red blood cell in leukemia and polycythemia vera have come to autopsy. The present paper reports the tissue C^{14} levels found in these patients.

METHODS

The patients had been given 100 microcuries (8.2 mg.) of glycine–2–C^{14} (methylene labeled) as described previously. Autopsies were carried out as soon as possible and in no case later than 12 hours after death. Samples of fresh tissue were taken in Patients 1, 3, and 4. In Patient 2 the samples had been placed in formalin for five to 10 minutes before the blocks were recut and tissue taken. The tissue was cut into approximately one gram samples and dried in vacuo. The bone sample was taken from the body of the sternum. The dried tissue was then combusted to carbon dioxide using a chromic-sulfuric acid oxidizing mixture (3). The carbon dioxide was precipitated as barium carbonate, the radioactivity of which was measured by the use of a vibrating reed electrometer and a 100 cc. ionization chamber operating at atmospheric pressure (4, 5).

RESULTS

The specific activity of the tissues is presented in Table I. Figure 1 shows the specific activity of the lungs, the myocardium, the kidney, and the liver of the four patients plotted on semilogarithmic paper as a function of time.

DISCUSSION

The table shows that within 57 days after administration the tissue concentrations in Patient 1 ranged from 0.04 dis./min./mg. of barium carbonate in the fat to 0.74 dis./min./mg. of barium carbonate in the spleen. If the fat is excluded from consideration, the range of specific activities in this patient was from 0.12 to 0.74 dis./min./mg. $BaCO_3$, or an approximately sixfold variation. It is interesting to note in this patient that the specific activity of the bone is near the average of all the tissues and that there are several tissues, particularly the myocardium, skeletal muscle, and the spleen, in which specific activity was considerably higher than that of the bone. In Patient 2 only five tissues were available, and these had been previ-

TABLE I

Tissue specific activities in dis./min./mg. $BaCO_3$

Autopsied at.....	Patient 1 57 days	Patient 2 105 days	Patient 3 152 days	Patient 4 526 days
Aorta	0.24		0.077	
Ilium	0.12		0.14	
Bone	0.37	0.15	0.35	0.038
Pancreas	0.40			0.038
Adrenal	0.30		0.103	0.02
Lung	0.44	0.300	0.129	0.044
Spleen	0.74		0.21	0.041
Liver	0.45	0.18	0.101	0.018
Bone marrow	0.33		0.137	0.047
Lymph node	0.27		0.123	
Kidney	0.42	0.25	0.087	0.026
Fat	0.04		<0.02	0.021
Myocardium	0.73	0.332	0.099	0.031
Skeletal muscle	0.614		0.42	0.081
Trachea			0.083	
Skin			0.014	0.179*
Stomach			0.045	0.043
Tumor				0.041
Bladder				0.041
Blood				0.078
Urine				0.005

* This result may be spurious; sufficient material to rerun this sample was not available.

[1] This study was supported by the Atomic Energy Commission.

[2] This has recently been confirmed by Hellman and associates (2).

ously slightly fixed in formalin so that although the data are probably reliable, the specific activities may be low. The tissues from this patient also show a comparatively small range of specific activities. The third patient shows a range of specific activities from a tissue in which no specific activity could be determined, the fat, to one in which the specific activity was 0.42 dis./min./mg. $BaCO_3$. Here also the skeletal muscle was the tissue with the highest specific activity. In Patient 4, who was autopsied 526 days after administration of the C^{14} labeled glycine, it is interesting to note that there is still a measurable amount of C^{14} present. It should be pointed out, however, that with the exception of skeletal muscle and the whole blood, the tissues are all close to the limit of resolution for the method of measurement of C^{14} which is employed (4, 5). Nevertheless, in each instance there was present a small, definite, and measurable quantity of activity; however, the actual numerical values are subject to considerable error at this level, and the value should not be regarded as highly precise.

Because all of these patients had lost considerable weight during the terminal phases of their illnesses, it is somewhat difficult to determine the precise amount of C^{14} present at autopsy; but in Patient 4 it can be estimated that approximately 1–2 per cent of the dose had been retained as late as 526 days. Figure 1 shows that for the four organs from which samples are available from all patients there appears to be a common component of elimination of C^{14}. As can be seen from the data on Patient 4, the values anticipated by extrapolation from the first three patients are considerably lower than those actually obtained at 526 days. This would indicate that there is another component of elimination having a half-time longer than 50 days.

From the data on the elimination of C^{14} in the breath, it was postulated that there should be another component of elimination from the body with a half-time of at least 90 days. Study of the long-term urinary C^{14} excretion shows that there is a component of urinary C^{14} excretion with a half-time of approximately 50 days (6). This is in close agreement with the anticipated value found from the tissue studies reported here and would indicate that instead of a fourth component of respiratory elimination of 90 days or greater there is present a renal excretory component of elimination having a half-time of 50 days. In addition, components representing approximately 2 per cent of the administered dose must be present having a half-time of elimination much greater than 50

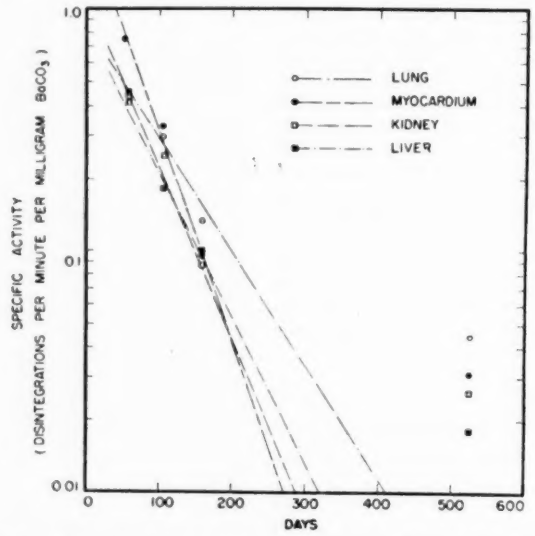

FIG. 1. SPECIFIC ACTIVITY OF LUNG, MYOCARDIUM, KIDNEY, AND LIVER

days. The mode of elimination of this 2 per cent may be either renal or pulmonary, but the specific activities are too small to measure with the instruments in current use.

SUMMARY AND CONCLUSIONS

1. The autopsy data on four patients given glycine–2–C^{14} are presented.

2. It has been shown that there is no marked concentration of the glycine–2–C^{14} in the bone and that with the exception of fat, which is very low in concentration, the tissue concentrations do not differ markedly.

3. There is a half-time of C^{14} elimination from the tissues which is approximately 50 days.

4. Autopsy of the fourth patient 526 days after administration indicates that there is at least one other component of elimination slower than 50 days.

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THE BASAL SECRETION OF PEPSIN BY THE HUMAN STOMACH [1]

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(Submitted for publication November 23, 1951; accepted January 8, 1952)

The basal secretion of the human stomach represents the juice secreted in the absence of all intentional and avoidable stimuli (1). The measurement of this phase of gastric secretion has been suggested by Bloomfield, Chen, and French as the simplest and most useful procedure in the study of clinical gastric physiology (2). These investigators demonstrated that the basal gastric secretion of hydrochloric acid was significantly greater in patients with duodenal ulcer than the values obtained in control subjects and in patients with gastric ulcer. Evidence regarding the basal secretion of pepsin however does not exist. Indeed there are considerable and serious deficiencies in our knowledge of the mechanism of pepsin secretion in man. This becomes especially clear if the meager existing evidence is contrasted with the extensive information which is now available regarding the mechanics of hydrochloric acid secretion (3). The present study was therefore designed to obtain quantitative evidence on the rate of secretion of pepsin by the human stomach under basal conditions, as the first step in a systematic study of the mechanics of this process. Subjects with a wide range of secretory activity, and a variety of clinical conditions, were studied. To extend the value of the information being collected, the simultaneous secretion of acid was also measured. The results obtained in control and peptic ulcer groups, and especially the correlation between the rates of enzyme and acid output, are presented in this report.

METHODS

Sixty-one subjects with a considerable range of gastric secretory activity were employed in this study. Of these, 21 were individuals with clinically active duodenal ulcer, confirmed either by the roentgen demonstration of an ulcer niche, or by surgical resection. Four patients had benign gastric ulcers proven by histological examination following surgical resection, five had gastric carcinoma, and five had addisonian pernicious anemia. The remaining 26 individuals, the control group, had no gastric disturbances or demonstrable lesions.

All subjects had subsisted on a liquid diet without any medication or sedation on the day preceding the experiments. All food was withheld following the evening meal. The subjects were then studied 18 hours later, in the fasting state. The spontaneous (non-stimulated) gastric secretion was collected for a total period of three hours by gastric intubation. Aspirations were performed every five minutes to insure completeness of collection, and the successive aspirates were combined into a single specimen for each hour. The volumes of these gastric collections were measured, and their acidities were determined by colorimetric microtitration using bromphenol blue and phenol red as indicators. Pepsin was determined by Bucher, Grossman, and Ivy's modification (4) of the Anson-Mirsky method (5), using lyophilized bovine hemoglobin powder in the substrate (6). Hourly rates of output of acid and pepsin were calculated from these volumes and concentrations.

RESULTS

The results of these experiments are recorded in Table I, where the hourly outputs of pepsin and acid are shown for each clinical category. For each group are given the range of values, the mean, and its standard deviation.

It is clear from these statistics that in the absence of obvious stimuli the duodenal ulcer group secreted three times as much pepsin as did the normal group. Although there is some overlap of the ranges of these two groups, the difference between their means is significant well below the 1% level ($t = 5.96$, $n = 45$, $P < 0.0001$). The means for the small number of gastric cancer and gastric ulcer cases are statistically identical with those for the normal group.

This table also demonstrates that the duodenal ulcer group secreted five times as much acid per hour as did the normal group. Here again, the difference between their means is significant at the 1% level ($t = 4.48$, $n = 45$, $P < 0.0001$), despite the overlap of their ranges.

When the hourly outputs of gastric pepsin are compared with the hourly outputs of acid in all

[1] This study was conducted with the aid of grants from the Altman Foundation and Wyeth, Incorporated.

TABLE I

Basal secretion of pepsin and acid in man

Clinical category	No. of cases	Hourly output					
		Pepsin (*Hgb units*)			HCl (*mEq*)		
		Range	Mean	SDm	Range	Mean	SDm
Control	26	0– 8,335	4,119**	±2,881	0– 1.67	0.61*	±0.50
Duodenal ulcer	21	2,914–35,050	14,241**	±7,142	0.14–10.66	3.30*	±2.93
Gastric ulcer	4	5,314–11,215	6,736	±2,954	0– 3.62	2.27	±1.37
Gastric cancer	5	0– 3,354	1,512	±1,080	0– 0.48	0.15	±0.17
Pernicious anemia	5	0	—	—	0	—	—

* For the difference of the means, t = 4.48, n = 45, P <0.0001.
** For the difference of the means, t = 5.96, n = 45, P <0.0001.

the individual subjects, a moderately high positive correlation ($r = +0.86$) is obtained. This correlation coefficient corresponds to a probability P-value of less than 0.1% ($n = 59$, $P < 0.001$). This relationship is illustrated graphically in the scatter diagram of Figure 1, where the average hourly output of pepsin is plotted against that of acid; the straight line has been fitted by the method of least squares.

DISCUSSION

Since the basal gastric secretion was defined by Lim (1) as the juice secreted by the stomach in the absence of all intentional and avoidable stimuli, the

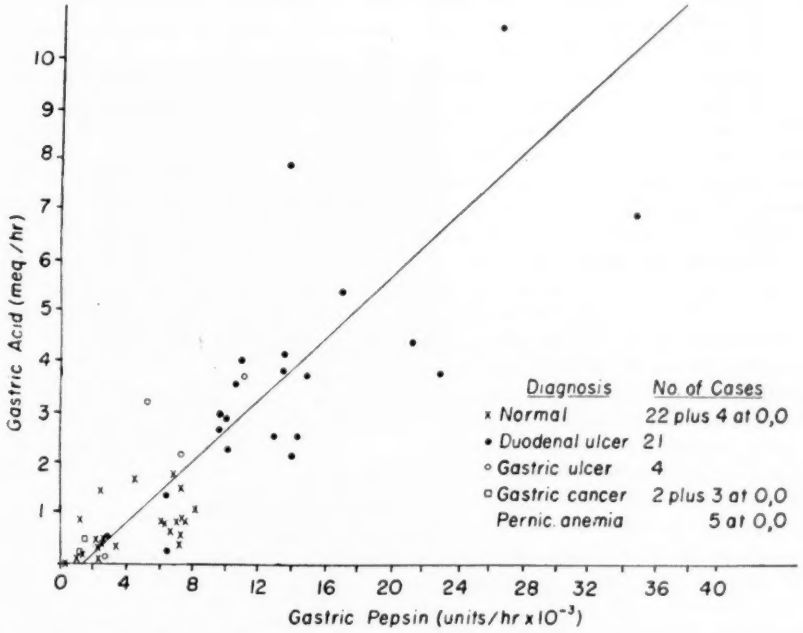

FIG. 1. SCATTER DIAGRAM SHOWING RELATION OF GASTRIC SECRETION OF PEPSIN TO ACID IN 61 SUBJECTS

As indicated in the legend, there are 12 points which fall at the origin (0,0) and are therefore not shown ($r = +0.86$, $P < 0.0001$).

effort was made in the present study to measure secretion under basal conditions by keeping the subjects' stomachs at rest for 18 hours before starting collection, and performing the experiments in an isolated room.

The results obtained indicate that there is wide variation in the level of peptic secretory activity in the resting human stomach, ranging from 0 to 8,335 units per hour in the control group, and from 2,914 to 35,050 units per hour in the duodenal ulcer group. Despite the range of values observed for each of the clinical categories studied, it is clear that on the average the duodenal ulcer group secreted three times as much pepsin as did the normal group. The situation in regard to pepsin is thus exactly similar to that already demonstrated by Bloomfield and his associates (*2*) for the basal secretion of acid, and which we have also confirmed in the present study.

Of great interest is the finding that there is a significant degree of correlation between the amounts of pepsin and the amounts of acid being secreted by the resting human stomach, acid and enzyme varying directly with each other. Since HCl and pepsinogen are derived from separate cell types of the gastric glands, the implications of this correlation are intriguing. They suggest that the peptic cells and the parietal cells are responding to some common influence in the basal state. This common influence may perhaps be the intensity of vagal stimulation, as has been maintained by numerous investigators, but no conclusive evidence for this exists at present. Hunt (*7*) also has recently observed a correlation of the amount of pepsin, secreted in response to histamine and to a meal, with the amount of acid secreted in response to these stimuli. He has suggested also that this is consistent with the hypothesis that some common factor controls the reactivity of the parietal and peptic cells, a factor which he believes may be vascular in nature. This correlation of acid and pepsin secretion suggests, also, that there is no significant qualitative difference in the character of the secretion occurring in the stomach of patients with duodenal ulcer as compared with that which occurs in the normal individual. Rather, it indicates only a quantitative difference in their respective levels of secretory activity, the relative outputs of acid and pepsin tending to be the same in both categories of subjects. The present evidence is thus consistent with the generally held, but by no means proven, concept that there is hypervagal activity in the stomach of the duodenal ulcer patient.

SUMMARY AND CONCLUSION

1. The basal secretion of pepsin and acid was measured for periods of three hours in 61 individuals, comprising a wide range of secretory activity and a variety of clinical conditions.

2. Twenty-one duodenal ulcer patients secreted, on the average, three times as much pepsin per hour as did the 26 individuals of the control group without gastrointestinal lesions. A small group of four gastric ulcer and five carcinoma patients fell within the normal range of secretory behavior.

3. Similarly, increased secretion of acid also occurred in the duodenal ulcer group in the basal state.

4. A high degree of positive correlation was demonstrated between the amounts of pepsin and of acid secreted per hour, suggesting that in the basal state the peptic and the parietal cells are responding to some common influence, possibly of vagal origin.

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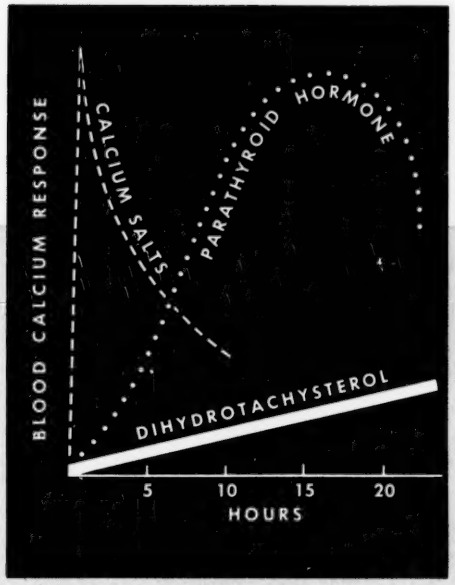

*Grollman, A.: Essentials of Endocrinology. Philadelphia, J. B. Lippincott Co., 1947, 2nd ed., p. 269.

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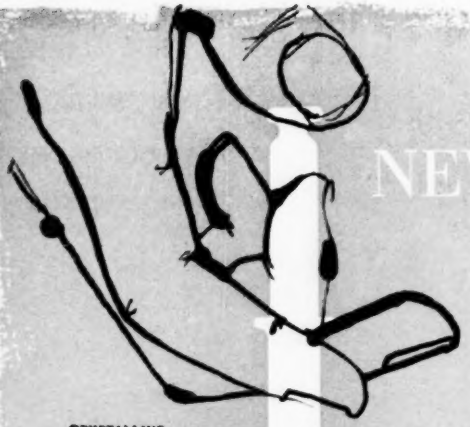

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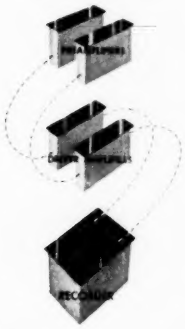

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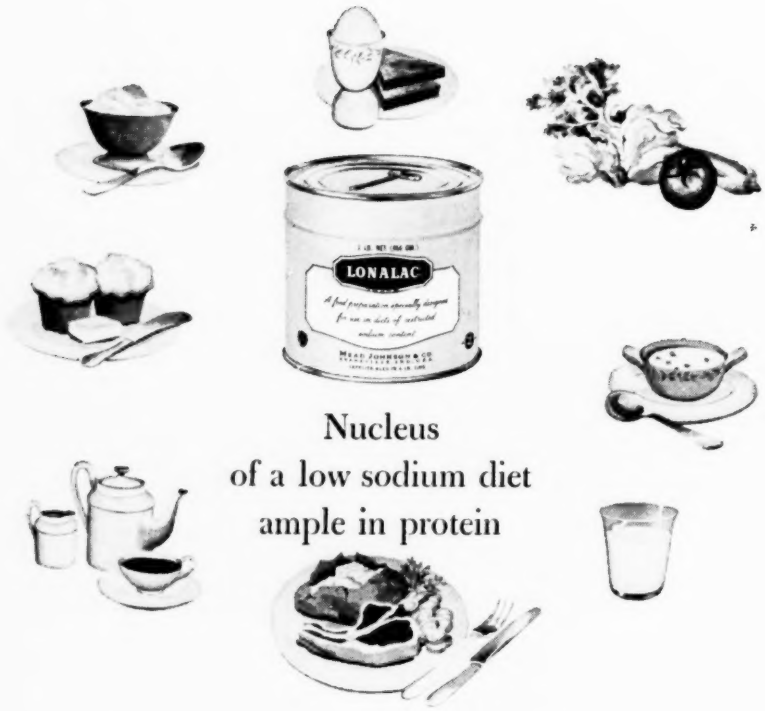

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Whereas most foods abundant in high quality protein contain large quantities of sodium also, Lonalac has a negligible 0.02%.

Nutritionally similar to whole milk, Lonalac can be used in any way in which milk is used—as a beverage, as a replacement for cream, or in soups, white sauces, breads, cakes, muffins, puddings and other appetizing dishes.

Used to replace milk, Lonalac can be combined with limited amounts of meat and eggs, and with low sodium foods, to provide nutritionally adequate diets containing as little as 200 mg. of sodium daily.

Flexible low sodium diet outlines and recipes employing Lonalac are available on request.

LONALAC is supplied in 1 and 4 pound tins.

MEAD'S

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DH-Alkaloids of Ergot Open Further Areas for Applied Research

Chemical research has made available a new set of pharmacologically interesting agents. This development evolved in two steps:

> *Fractionation* of the substance known as "ergotoxine" yielded three separate and distinct alkaloids: ergocornine, ergocristine and ergokryptine.
>
> *By hydrogenation* dihydro-derivatives have been produced. These compounds present a new realm of pharmacologic properties and clinical potentialities.

PHARMACOLOGIC PROPERTIES

Studies to-date with an equi-proportional mixture of the three dihydro-alkaloids (available as Hydergine*) have disclosed the following actions:

(a) *Central Actions* —
  (1) sedative effect
  (2) dampening of impulses from vasomotor center

(b) *Vagal Action* — producing bradycardia

(c) *Peripheral Action* — adrenergic blockade

Further observations show that in therapeutic dosage range the DH-ergot-alkaloids selectively inhibit sympathetic vaso-constrictor impulses without modifying other adrenergic function and the response to vasodilator mechanisms.

CLINICAL ASPECTS

Studies, thus far reported, demonstrate the value of Hydergine in therapy of peripheral vascular diseases and hypertension. (See information below.) The possibility of therapeutic value in various other conditions is still open for final evaluation; for instance in:

(a) trench foot, frost-bite, chilblains
(b) angina pectoris
(c) toxemia of pregnancy
(d) postoperative gastro-intestinal atony
(e) pheochromocytoma
(f) potentiation of barbiturate sedation

For additional information, contact the Research Department at address given below.

Articles on hypertension and peripheral vascular diseases report the following clinical benefits with Hydergine:

> *Hypertension* — lowers blood pressure; relieves headaches, nervousness, dizziness (by improving cerebral circulation); decreases heart-load by producing bradycardia.
>
> *Peripheral Vascular Diseases* — improves collateral circulation; helps reduce tissue necrosis; may be used in conjunction with sympathectomy.

Reprints and abstracts of published reports dealing with these indications are available on request; write to:

Sandoz Pharmaceuticals

DIVISION OF SANDOZ CHEMICAL WORKS, INC.
68 CHARLTON STREET, NEW YORK 14, N. Y.

*1 cc ampul, Hydergine contains: dihydroergocornine, dihydroergocristine, dihydroergokryptine, 0.1 mg. of each.

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